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A STUDY OF SYSTEMIC INFECTION BY DOWNY MILDEW OF SOYBEAN WITH SPECIAL REFERENCE TO SYMPTOMATOLOGY, ECONOMIC SIGNIFICANCE AND CONTROL

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Of the 14 different diseases of a parasitic nature, which have been reported by the present authors (10, 12) as occurring on soybeans in south-western Ontario during the past few years, none has been more widespread or consistent in its occurrence on susceptible varieties than downy mildew (6) caused by *Peronospora manshurica* (Naoum.) Syd. In conjunction with soybean seed treatment studies of a general nature which were carried out at the Harrow laboratory over a considerable period of time (10, 13), the effect of seed treatment as it related more particularly to the downy mildew disease was also investigated. In addition, studies were made of the systemic type of infection, especially with regard to symptomatology, incidence and spread, and seed-transmission of the disease. A review of these various phases of investigation is embodied in the present paper.

HISTORY, DISTRIBUTION, AND AETIOLOGY OF THE DISEASE

In 1921, Miura (22) briefly described a downy mildew occurring on soybeans in Manchuria, the causal organism of which was identified as *Peronospora trifoliorum* de Bary var. *manshurica* Naoumoff (23). In 1922, according to Haskell and Wood (9), "soybeans were attacked for the first time recorded in the United States by a downy mildew due to a *Peronospora* sp." In 1923, Lehman and Wolf (19, 20) encountered the disease in North Carolina and, believing the associated organism to be a hitherto undescribed species of *Peronospora*, proposed for it the new name *P. sojae* Lehm. and Wolf. While the manuscript of their paper was in press, they received a copy of Miura's publication in which the cause of the apparently identical disease in Manchuria had been ascribed to *P. trifoliorum* var. *manshurica* Also, it had come to the attention of Lehman and Wolf that, in 1923, *Gäumann* (7) had raised *P. trifoliorum* var. *manshurica* to specific rank, the downy mildew organism thereupon becoming *P. manshurica* (Naoumoff) Sydow. In 1926, Wolf and Lehman (27) compared mildew-infected soybean plants from North Carolina with similarly infected specimens from Manchuria and found the disease to be identical on both. The causal organism

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as well agreed morphologically with Gäumann's species *manshurica*. Thus, on *a priori* grounds, the American workers recognized the validity of the combination *P. manshurica*, and *P. sojae* was relegated to synonomy.

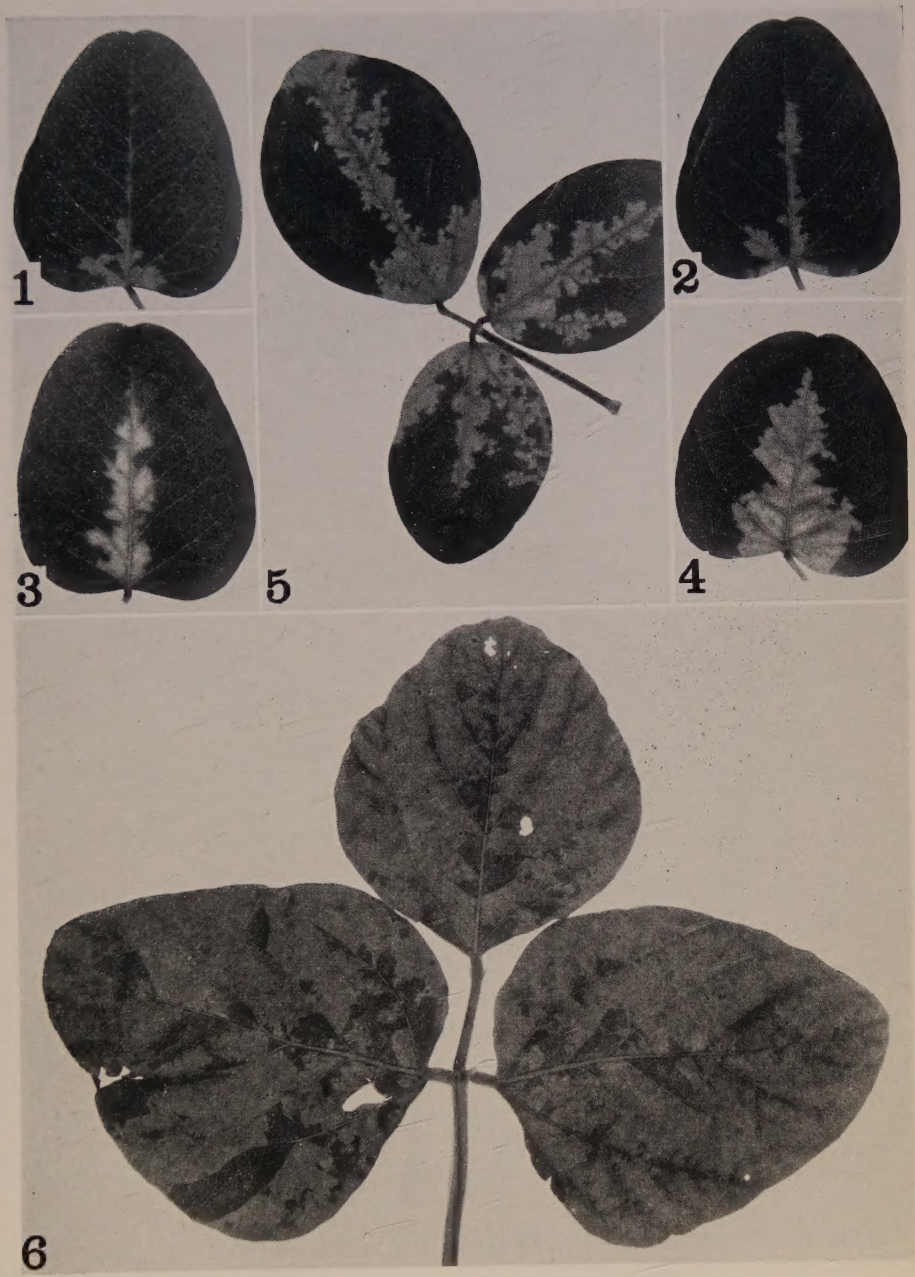
Until 1942, on this continent, soybean downy mildew was considered to be strictly a foliage disease. In that year, however, Johnson and Lefebvre (16) described seed encrusted with oospores of *P. manshurica*, but these investigators made no reference to the systemic nature of the disease. So far as the present writers are aware, the earliest statement regarding the latter was made by Abramoff (1) who, in 1928 and 1929, recorded that downy mildew (*Peronospora manshurica*) may either produce localized infection, in which case the damage is insignificant, or systemic infection, which causes a noticeable stunting of all the aerial organs of the host, with a consequent considerable reduction in yield. In 1944, Atkinson (3) in commenting on an outbreak of downy mildew on soybeans in North Carolina stated, "some plants were so thoroughly infected that the disease appeared to be systemic". In 1945, the present authors (10) interpreted careful observational evidence as indicating that seed infected by downy mildew gave rise to systemically-infected plants. Definite confirmation of this observational evidence based on the microscopic examination of seedlings originating from seed encrusted with oospores of the fungus was recorded in 1946 (18). In the same year, Jones and Torrie (17) also reported that seed encrusted with oospores of *P. manshurica* when planted in the greenhouse gave rise at times to seedlings with lesions apparently connected by mycelium in a systemic infection.

The disease has been reported from various parts of Canada (6), from practically every state in the United States where soybeans are grown[1], and from such widespread other locations throughout the world as India (5), China (25), the Asiatic Far East (1), Sweden (21), and South Africa (2). Thus, it would seem probable that the disease is co-extensive with the cultivation of the crop.

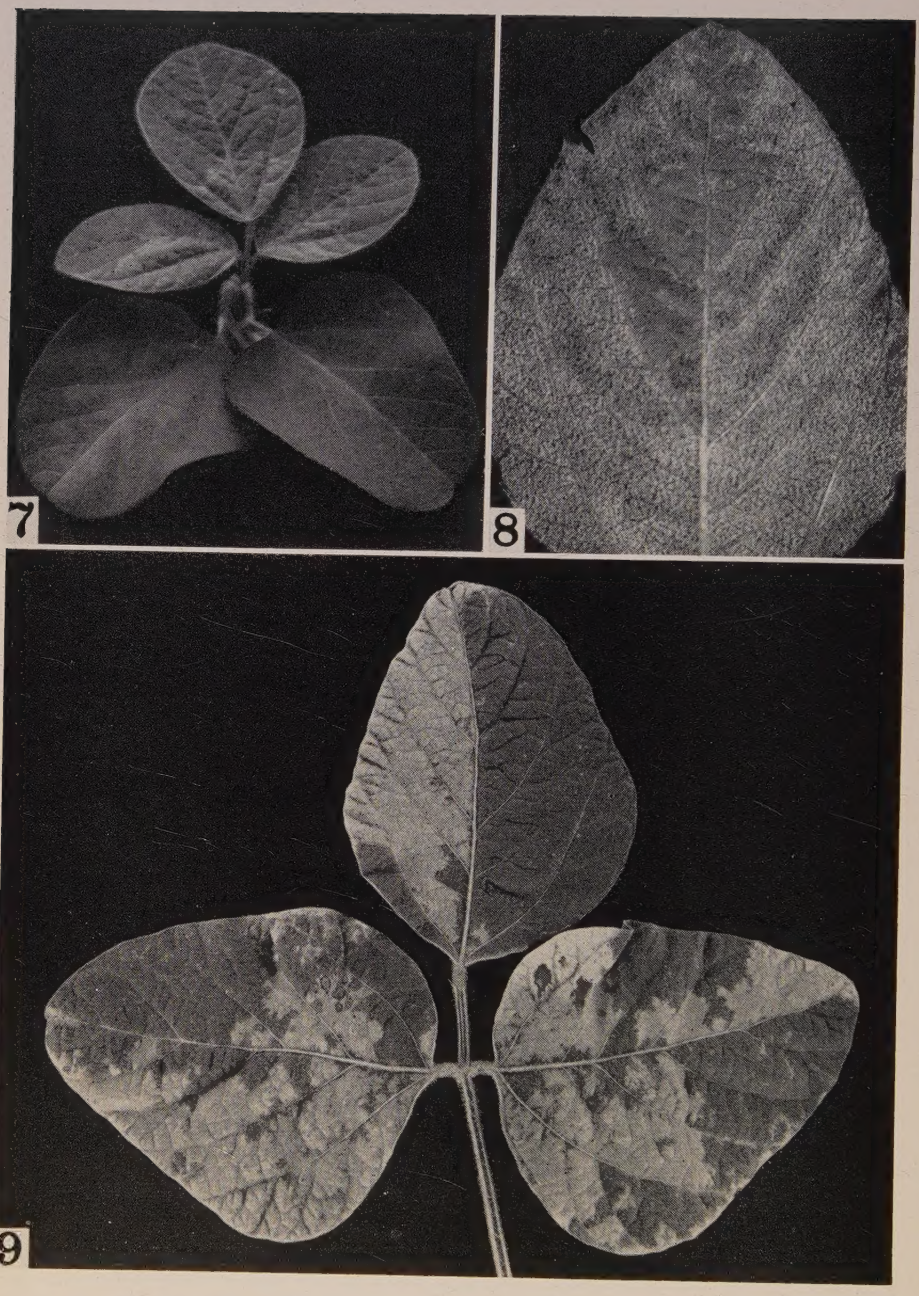
MATERIALS AND METHODS

The investigations herein reported were carried out in the laboratory, in the greenhouse, and in the field. The laboratory phase involved the microscopic examination of stained sections, not only of mildew-infected seed, but also of seedlings and plants originating from such seed at progressive stages in their development. For microscopic examination, razor sections of subject material were boiled in lacto-phenol, with acid fuchsin added. Mildew-infected seed planted in the greenhouse furnished material both for studies of symptom expression and for determinations of incidence of disease on a numerical basis. Field studies, carried out in 1946, 1947 and 1948, followed the selecting and planting of many thousands of mildew infected seeds of susceptible varieties, and centred primarily on an attempt to evaluate the effect of seed treatment on emergence, incidence and severity of disease, and yield. Detailed information in connection with the field-plot technique will be given in a later section.

[1] The present authors have consulted over 50 references in the literature, which, in the aggregate, report the occurrence of the downy mildew disease in some 19 different states of the United States. Although these references cover the 28-year period from 1922 until 1950, the majority of them date from about 1940.



Foliar symptoms of systemic infection. FIGURES 1, 2, 3, AND 4.—Unifoliate or first-formed true leaves showing progressive stages of infection. FIGURE 5.—Young trifoliate leaf showing pattern of infection. FIGURE 6.—Mature leaf showing mosaic-like mottle due to colour contrasts between intercostally-occurring "green islands" and surrounding leaf tissue.



Foliar symptoms of systemic infection. FIGURE 7.—Sixteen-day-old seedling showing incipient infection of both unifoliate leaves and leaflets of first trifoliate leaf. FIGURE 8.—Mature leaf showing under-surface covered (except for "green-island" areas) with fungus in sporulating stage. Figure 9.—Mature leaf showing exaggerated case of colour contrast as result of infection.

SYMPTOMATOLOGY

On Seed

If seed samples of certain of the more susceptible varieties of soybeans grown in Ontario, such as A. K. Harrow, Capital, Hawkeye, Harly, and Harman, are examined even casually, almost invariably attention will be attracted to a varying number of seeds which lack the lustre and smooth-coatedness characteristic of the healthy seed of these and other varieties. If these particular seeds are examined more closely, it will be noted that they are more or less covered with a powdery-appearing, milk-coloured encrustation, which is quite firmly adherent to the seed coat (Figure 19). If a scraping of the encrustation is examined microscopically, it will be found to consist of a closely cohering mass of the resting spores or oospores of the downy mildew fungus (Figure 15). As mentioned above, Johnson and Lefebvre (16), in 1942, were the first to record observations in this connection, but an announcement as early as 1924 by Wolf and Lehman (26) had proclaimed definitely the seed-borne nature of the disease. Illustrations of mildew-encrusted seed were shown by Petty (27) and by Johnson and Koehler (15) in 1943.

On Seedlings and Developing Plants

(a) *Systemic Infection*—When oospore-encrusted seeds of the more susceptible varieties are planted either in the greenhouse or out-of-doors, the majority of them give rise to normal, healthy plants, a few fail to produce seedlings at all, and a usually small though variable number of them produce systemically-infected plants. The effect of *P. manshurica* on the plants appears to vary widely. Some infected seedlings develop into unthrifty, dwarfed plants which are soon overshadowed in the row and which, unable to compete with healthy, neighbouring plants, die earlier in the growing season, but not before many of them have produced an abundance of conidia which infect surrounding, healthy plants. Other infected seedlings remain alive but exhibit a markedly spindly type of growth throughout the season. This type of plant may, however, produce pods, although the latter, reduced both in number and size, often contain not more than one or two viable seeds. Still other infected seedlings grow almost normally, except for certain foliar symptoms, and produce at maturity a quota of seed which, though infected, may, quantitatively, almost equal that matured by a healthy plant.

The first visual evidence of systemic infection is indicated by foliar symptoms when the seedling, about two weeks old, has formed the pair of unifoliate or first true leaves. On the latter the presence of the disease is indicated by a clearly defined, lighter green area which appears on the lamina near the point of its juncture with the petiole (Figure 1). Infection may progress along the mid-vein in a serrated pattern (Figures 2, 3, 4) or spread fanwise from the point of initial entry into the lamina (Figure 5). Occasionally, an initial area of infection may appear on a leaf at a point remote from the juncture of lamina and petiole. The disease next involves the trifoliate leaves (Figures 5, 7) as they are formed, in a manner closely similar to that described for the unifoliate leaves; but all trifoliate leaves do not necessarily exhibit visible evidence of infection. Finally, the latest formed leaves at the top of the plant show evidence of the presence of the disease. These top leaves, often attracting attention by their gray-green

colour, may present also a distinctly mottled or mosaic-like appearance (Figures 6, 9), the latter being intensified at times by a more or less rugose condition of the leaf surface. The mottle and rugosity are due to the presence of "green islands" which occur intercostally on the leaf.

About a week to 10 days after visible evidence of infection, the fungus starts fruiting on the under-surface of the lighter green areas on infected leaves, first on the unifoliate leaves close to the ground and then on the trifoliate leaves progressively upward on the stalk. Often the entire under-surface of a leaf or leaflet, except the "green-island" areas (Figure 8), may be covered by the fungus, which produces conidia most prolifically. The usually grayish fungus mat may at times exhibit a faintly purplish tinge. By way of contrast with *P. manshurica*, it is interesting to note that, as Gäumann (8) points out, under optimal conditions of temperature and humidity, the conidiophores of the downy mildew, *Peronospora brassicae*, break through the lower surfaces of the cotyledons of cabbage seedlings before any other definite symptoms of disease are apparent. The same is true for the downy mildew, *P. cheiranthi*, of the wallflower, *Cheiranthus cheiri*.

(b) *Local Infection*—Early local infection by the downy mildew fungus is characterized by the occurrence of indefinite chlorotic flecks (Figure 10) on the upper surface of affected leaves. As the mycelium of the fungus progresses through the leaf tissues, the flecks enlarge and change to well-defined, grayish-brown to dark-brown or reddish-brown lesions (Figure 11) surrounded by a more or less distinct halo. Meanwhile, beneath the lesions, grayish or faintly purple-coloured wefts of conidiophores develop on the under-surface of the leaves (Figure 12).

Often, in a stand of soybeans, a number of plants within an area will show heavy local infection, the circumstances being strongly suggestive of some proximal and abundant source of inoculum. If search is made, such source is frequently found to be a more or less inconspicuous, systemically infected plant of a type described above.

LABORATORY AND GREENHOUSE STUDIES

Evidence as to the systemic nature of mildew infection was sought in two ways, namely, by microscopic examination both of seeds and of plant parts, and by comparing the incidence of mildew-infected plants derived from healthy and from mildew-encrusted seed.

Microscopic evidence of systemic infection

Microscopic studies began with an examination of the seed-coat and embryonic tissues of mature, oospore-encrusted seeds. Sections were prepared both from the excised hypocotyledonary axis and from the cotyledons themselves. For studies of seedlings in their earliest stages, mildew-encrusted seed was germinated either in soil or on moist blotting paper in Petri plates and sections were cut from developing hypocotyledonary and epicotyledonary tissues. As seedlings developed into older plants, either in the greenhouse or in the field, sections were cut from roots and stems and from leaf-, flower-, and fruit-parts, in a sequence related, when possible, to visible evidence of the presence or spread of infection.

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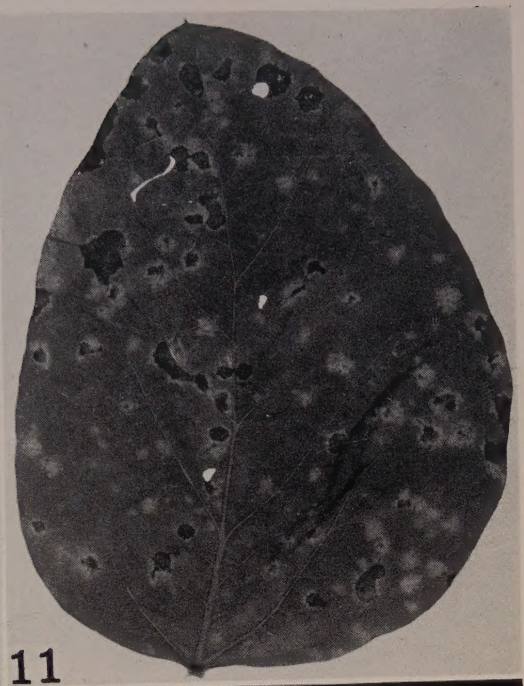

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Foliar symptoms of local infection. FIGURE 10.—Early stage of multiple local infection on upper leaf surface. FIGURE 12.—Lower surface of leaf showing early local infection. Mycelium has progressed through leaf tissues and is now producing visible wefts of conidiophores. FIGURE 11.—Later stage of local infection showing necrotic areas surrounded by halo on upper leaf surface.

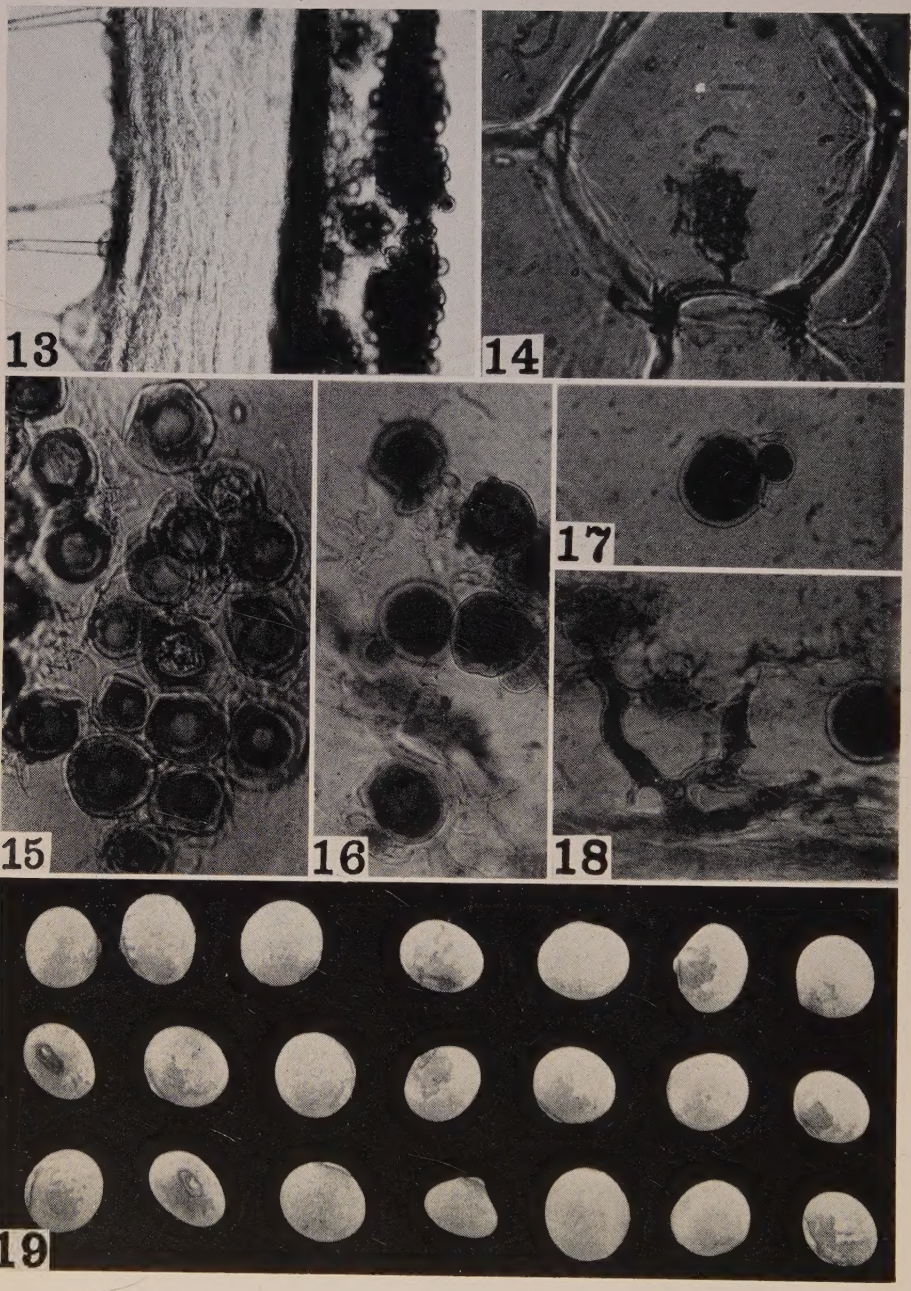

FIGURE 13.—Transverse section through maturing pod showing endocarp lined with mass of oospores (× 80). FIGURE 15.—Cluster of typical oospores (× 320). FIGURES 16 and 17.—Oospheres with attached antheridia as observed on endocarp (× 320). FIGURE 14.—Haustorium in pith cell of hypocotyl of 9-day-old seedling (× 400). FIGURE 18.—Mycelium (× 320). FIGURE 19.—Mature seed encrusted with oospores (× 1½).

In the case of mature seed, while sections show an abundance of oospores on the surface of the seed coat and considerable, though fragmentary, mycelium within the palisade and "hour-glass" cells comprising the seed coat, nevertheless, the presence of the fungus in embryonic tissue is much less readily apparent. No evidence of the presence of the fungus in the excised hypocotyl and plumule has been obtained and in only rare instances has mildew mycelium been detected in cotyledonary tissue.

As stated above, in the section on symptomatology, the first visual evidence of systemic infection of seedlings is indicated by the appearance of lighter-green areas on the lamina of the unifoliate or first true leaves near the point of its juncture with the petiole. The earliest appearance of this particular symptom was noted on a 14-day-old, greenhouse-grown seedling. The earliest microscopic evidence of systemic infection was found in sections taken from the hypocotyl of a 9-day-old, greenhouse-grown seedling. The hypocotyl in this particular seedling had not yet lengthened or straightened sufficiently to have raised the cotyledons above the ground, and the part from which the sections were cut was visible above ground only as a green loop. If this seedling had been selected for examination a day or two previously, it is possible that the presence of the fungus could have been established just so much earlier. This surmise is based on the fact that by the ninth day, mycelium and haustoria were both present in abundance in the tissues of the hypocotyl at a point at least $\frac{3}{4}$ in. from the point of attachment of the cotyledons. The type of haustorium observed in cells of the pith is shown in Figure 14. In 1941, Ikata and Yamauti (14), in referring to the haustoria of *P. manshurica* in soybean, commented "the young haustoria twist, coiling at maturity to resemble intestines".

In additional seedlings from 10 to 16 days old, further evidence was adduced from microscopic examination, not only that the fungus was present and spreading in the rapidly developing tissues of the host, but that relatively extensive internal infection could precede by several days any external manifestation of the disease. With regard to rate and extent of spread by the fungus within the plant tissues, the following observation was made on a greenhouse-grown seedling. On February 7, 1948, the first faint symptoms of systemic infection became apparent on one of the unilateral leaves. Eight days later, the first trace of infection could be detected on the lamina of one of the leaflets of the first trifoliate leaf. Measurement disclosed that in the 8-day period the fungus had progressed through 95.28 mm. of stem and petiole tissue.

When older plants were examined, it became apparent also that extensive internal infection of leaves, resulting in the foliar symptoms described above, could precede, by some considerable time, the appearance of and sporulation by the fungus on the ventral surface of the leaf. A single typical case may be cited as illustrative of these several points. On July 15, 1948, a 55-day-old plant, derived from an oospore-encrusted seed and growing outdoors in the laboratory experimental plots, was selected for microscopic study. This plant had reached the ninth-leaf stage. The very small, ninth (latest-formed) leaf was just unfolding from the bud, the eighth, though expanded, was still relatively small, and the seventh was about two-thirds developed. The internodes in this apical portion of the stem were relatively short. Macroscopically, there was nothing in the

appearance of these three uppermost leaves to suggest the presence of mildew. The leaflets of the sixth, fifth, and fourth leaves showed, however, typical symptoms of the disease, which ranged from the less distinct, lighter-green areas on the sixth leaf to the conspicuous, grayish-green discolouration and mosaic-like mottle on the fourth (Figure 6). Examination with the dissecting microscope failed to disclose any sign of the fungus externally on the under-surface of these three leaves. The third, second, and first leaves towards the base of the stem showed marked mildew symptoms and the fungus was sporulating abundantly over the entire under-surface of each leaflet, except where "green-islands" occurred (Figure 8). For microscopic examination, sections were cut from the petioles and laminae of the three youngest leaves, from successive internodes down the stem, and from several locations on the root. Evidence of the presence of the fungus was found in all sections except those taken from the region of the ninth and the eighth leaves, thus indicating almost complete systemic infection in both aerial and underground parts of the plant. The observations recorded for this plant were confirmed in whole or in part by the similar, systematic examination of other selected specimens.

From the practical standpoint, one of the most important aspects of systemic infection of the soybean plant is how and to what extent the seed may be implicated. During the formative period of the latter, pods from selected plants were examined microscopically. Again, a single illustrative case may be cited. On September 5, 1948, a number of pods, still green in appearance though almost fully developed, were collected from a systemically infected plant. None of the pods showed any external symptoms of mildew. Transverse sections through a seed were made from several of these pods. An examination of these sections readily disclosed that the endocarp or inner pod wall was lined with, and the outer integument or seed coat was more or less completely enveloped by not only the mycelium (Figure 18) but also the sexual organs (antheridia and oogonia, Figures 16 aud 17) and oospores (Figures 13 and 15) of *P. manshurica*. Thus, it was clear that the sexual stage in the life history of the fungus was being completed inside the pod in contact with the seed. These observations made it readily understandable how soybean seed can become invested with masses of the resting spores of the downy mildew fungus. As for the seed itself, mycelium was noted, in these particular sections, in the seed coat but not in the embryonic tissues. The mycelium in the seed coat was fragmentary and obviously in a stage of senescence. No haustoria were observed. The latter had probably ceased to function as the seed approached maturity and may possibly have disintegrated.

Evidence of systemic infection from planting of oospore-encrusted seed

A number of experiments of a preliminary nature carried out in the greenhouse had indicated that oospore-encrusted seed would give rise to an appreciable percentage of systemically infected plants, whereas presumably healthy seed would rarely or never do so. Details in connection with these exploratory experiments will not be elaborated here; rather attention will be directed to the findings of an experiment carried out on a larger scale.

In the autumn of 1946, the oospore-encrusted fraction in seed lots of the varieties A. K. Harrow and Harman were separated from the healthy

seeds by hand selection. A portion of the healthy and of the mildewed seed of each variety was stored over winter in an unheated warehouse, while corresponding portions were kept during the winter in the heated laboratory. On March 7, 1947, a total of 32 eight-inch pots containing composted soil were planted with seeds of each variety, representing the healthy and diseased categories which had been overwintered under the two different storage conditions. Fifteen seeds were planted in each pot. The plants were kept under close observation and diagnoses based on visual symptoms were confirmed by microscopic examination of stained sections. The results of the experiment are summarized in Table 1.

TABLE 1.—GERMINATION AND INCIDENCE OF SYSTEMIC MILDEW INFECTION IN TWO SOYBEAN VARIETIES IN RELATION TO PRESENCE OR ABSENCE OF OOSPORES ON SEED AND TO DIFFERENT OVERWINTERING STORAGE CONDITIONS

Variety	Warm Storage						Cold Storage					
	Healthy seed			Mildewed seed			Healthy seed			Mildewed seed		
	G*	H*	M*	G	H	M	G	H	M	G	H	M
A. K Harrow	83.3	83.3	—	88.3	64.2	35.8	93.3	98.3	1.7	95.0	75.5	24.5
Harman	90.0	90.0	—	84.9	92.1	7.8	92.0	92.0	—	90.0	63.0	37.0

* G, H, and M = Total germination, healthy plants, and systemically infected plants, respectively.

So far as the main interests of this paper are concerned, the point of outstanding significance, as indicated by Table 1, is that healthy seed gave rise almost without exception to healthy plants, whereas oospore-encrusted seed produced systemically infected plants in relatively high proportions, e.g., 24.5, 35.8, and 37.0 per cent. Curiously enough, mildewed A. K. Harrow seed gave higher germination than healthy seed of the same variety under both conditions of storage. The tendency in the case of Harman was just the opposite to this. The two varieties, it will be noted, also reacted oppositely in the matter of incidence of mildewed plants. Warm-stored, mildewed Harman and A. K. Harrow seed gave rise to 7.6 and 35.8 per cent systemically infected plants, respectively. In the case of the cold-stored seed of the two varieties, however, Harman gave rise to 37 per cent and A. K. Harrow to only 24.5 per cent systemically infected plants. Thus, there is the indication that both the variety of seed and the conditions of storage may be factors modifying the incidence of systemic mildew infection.

Evidence as to the fungus being located primarily in the seed coat

In a preceding section it was pointed out that only on rare occasions did microscopic examination ever reveal the presence of the mildew pathogen in embryonic tissues of the seed. In a later section it will be shown that the disease is readily amenable to control by seed treatment. These two circumstances suggested that the pathogen might be carried principally in the seed coat To investigate this possibility further, the following experiment was carried out. On February 6, 1951, 400 oospore-encrusted seeds were selected from a seed lot of the variety A. K. Harrow, which, since the completion of harvesting operations in the autumn of 1950, had been stored over winter in the laboratory. The 400 seeds were placed on moist blotting paper in Petri plates where they were left for 24 hours. At the end of that

period, the seed coats were removed from 200 of the seeds and the latter were planted in the greenhouse on February 7 in composted soil in 8-inch pots, 20 seeds per pot. The other 200 seeds, with the seed-coats intact were similarly planted. By February 28, 6.4 per cent of the population of plants derived from the seed with the seed coats left intact showed systemic infection. In contrast, not a single systemically infected plant appeared in the population derived from the seed from which the seed coats had been removed. These results add confirmation to the view that the pathogen is carried primarily in the seed coat.

FIELD STUDIES

Effect of treatment of oospore-encrusted and of healthy seed on stand and yield

In 1946, 1947, and 1948, the effect of treatment on mildew-infected seed was investigated in trials on a field-plot scale. For the 1946 trials, not enough oospore-encrusted seed could be obtained from any individual lot of seed grown in 1945 but, from specimen lots supplied by growers and distributors of the district, an adequate amount of both A. K. Harrow and Harman was obtained. During 1946, A. K. Harrow plants growing in the laboratory experimental plots had been heavily infected with mildew and these plants furnished all the infected seed that was required for the 1947 trials. During the winter of 1947, in the course of a district survey of farm-stored soybeans, a lot of A. K. Harrow was found in which the oospore-encrusted fraction reached almost 11 per cent. This seed-lot was selected as the source of a ready supply of infected seed for the 1948 trials. Each year, except 1948, healthy seed selected from each lot furnishing mildew-infected seed was also treated and planted. The protectants used were Spergon in all three years and Fermate also in 1947. Spergon was given primary consideration because, over the years in general seed treatment tests (11), it had given consistently better results.

Each year, prior to planting, the soil, a Fox sandy loam, received an application of a 2–12–10 commercial fertilizer applied broadcast at the rate of 250 lb. per acre. All planting was done by hand in a randomized, 5-replicate design, the test unit within each plot comprising duplicate rows 30 ft. long. The rows, spaced 30 in. apart, were planted at the rate of 30 lb. per acre, this rate having been found by test to give maximum yield for the type of soil on which the experiment was being conducted.

Each year, counts made three weeks after planting, and subsequently, furnished the data on emergence and survival of seedlings. Except for hand weeding, cultural treatment of the plots conformed closely with accepted field practice for the crop. In harvesting, the plants were cut near the base of the stem with pruning shears, assembled in bundles, and threshed by means of a portable, power-driven, rod-row thresher. All weighings were made immediately after threshing and the yield data were examined statistically. The results of the tests from 1946 to 1948, inclusive, are summarized in Table 2.

As Table 2 shows, treatment of mildew-encrusted seed with the two protectants increased emergence in every instance but one, the exception being in 1946 when emergence from treated A. K. Harrow seed was 2.8 per cent less than from a lot of the same seed untreated. The maximum for increased emergence was 20.6 per cent (Harman, 1946). In two of the

TABLE 2.—EMERGENCE, INCIDENCE OF SYSTEMIC INFECTION, AND YIELD IN RELATION TO TREATMENT AND NON-TREATMENT OF HEALTHY AND OOSPORE-ENCRUSTED SEED OF TWO SOYBEAN VARIETIES

Variety	Treatment		1946						1947						1948		
			Mildew-encrusted seed			Healthy seed			Mildew-encrusted seed			Healthy seed			Mildew-encrusted seed		
	Material	Rate	Em.* %	Syst.* inf. %	Bu. per acre	Em. %	Syst. inf. %	Bu. per acre	Em. %	Syst. inf. %	Bu. per acre	Em. %	Syst. inf. %	Bu. per acre	Em. %	Syst. inf. %	Bu. per acre
A. K. Harrow	Spergon	3 oz. per bu.	89.3		26.58	94.1	0.1	26.25	94.2		26.78	84.6		24.78	90.6	0.6	40.6
	Check		92.1	5.4	26.50	84.3	0.1	26.11	88.6	.81	24.41	85.7		25.61	80.1	7.0	38.1
	Diff. due to treatment		−2.8	5.4	.08	9.8		.14	5.6	.81	2.37	−1.1		−.83	10.5	6.4	2.5
Harman	Spergon	3 oz. per bu.	82.6	1.0	29.81	65.7		28.38									
	Check		62.0	12.9	22.36	56.8		27.91									
	Diff. due to treatment		20.6	11.9	7.45	8.9		.47									
A. K. Harrow	Fermate	2 oz. per bu.							90.1		25.45						
	Check								88.6	1.7	24.41						
	Diff. due to treatment								1.5	1.7	1.04						
Difference required for significance (19 : 1)					2.78			2.78			2.78			2.78			2.78

* Each year, the exact number of all seeds planted was known; thus, it was possible to express emergence and incidence of systemically infected plants on an absolute percentage basis.

three trials in which healthy seed was treated, increases in emergence were recorded for both varieties in 1946 (A. K. Harrow 9.8 per cent, Harman 8.9 per cent), but, in the 1947 trial, germination of A. K. Harrow seed was 1.1 per cent less than that of the same seed untreated. In regard to the relationship between the incidence of systemic infection and the disease potentiality of the seed, two points stand out clearly: (a) that oospore-encrusted seeds give rise consistently to systemically infected plants, whereas healthy seeds only rarely do so, and (b) that seed treatment virtually eliminates systemic infection. Despite this latter fact, however, in only one of the five trials in which treated, mildew-encrusted seed was involved, was there a significant increase in yield, namely, the 7.45-bu.-per-acre increase from the treated Harman seed in 1946.

Oospores as possible overwintering agents of the disease

A point which presented itself for consideration in these studies was the possible role that the oospores of *P. manshurica* might play in the perpetuation of the disease from one season to the next. As mentioned above, oospores are formed in great abundance on the inner wall of pods produced by systemically infected plants. During September and October, 1946, pods from healthy and from systemically infected plants of the varieties A. K. Harrow and Harman were obtained. They were temporarily stored in a cool basement until November 4, on which date they were shelled, the empty pods and seeds being packaged separately and the packages then being buried to a depth of about 12 in. in a quantity of soybeans in bulk storage in an unheated warehouse. On May 23, 1947, 125 healthy A. K. Harrow seeds were planted in contact with 125 healthy pods of the same variety, in a 30-foot row. In an adjacent row the same number of healthy seeds was planted in contact with a corresponding number of pods obtained from systemically infected plants of the variety. In two contiguous rows, the scheme was duplicated with the Harman material. Each planting was replicated five times in a randomized design. The results are summarized in Table 3.

TABLE 3.—EMERGENCE, INCIDENCE OF SYSTEMICALLY INFECTED PLANTS, AND YIELD IN RELATION TO THE PLANTING OF HEALTHY SEED IN CONTACT WITH HEALTHY AND OOSPORE-LINED PODS, RESPECTIVELY

Variety	Planting combination	Emergence, %	Incidence of syst. inf'n. %	Yield bu. per ac.
Harman	Healthy pods and healthy seed	91.8	0.0	29.04
	Mildewed pods and healthy seed	88.0	1.6	24.88
	Difference	3.8	1.6	4.36
A. K. Harrow	Healthy pods and healthy seed	80.9	0.0	29.42
	Mildewed pods and healthy seed	78.4	0.3	26.23
	Difference	2.5	0.3	3.19
Difference required for significance (19:1)				2.78

As Table 3 shows, in neither variety was emergence from seed planted in contact with healthy pods appreciably higher than that from seed planted in contact with pods bearing the oospores of the fungus. In addition, it will be noted that in only a few instances did healthy seed planted in contact with infected pods give rise to systemically infected plants. The percentages, 1.6 for Harman and 0.3 for A. K. Harrow, represent an incidence of only 9 and 2 systemically infected plants, respectively, from 625 healthy-seed, mildewed-pod plantings for each variety. In view of these results, it is most surprising to note that in the case of both varieties, healthy seed planted in contact with healthy pods gave significantly higher yields than healthy seed planted in contact with infected pods.

DISCUSSION

Earlier in the present paper it was mentioned that some 50 references relative to the occurrence and distribution of soybean downy mildew in the United States had been consulted. Although the majority of these references are little more than citations as to date and location of the appearance of the disease, some of them do attempt to evaluate the significance of the disease as a possible limiting factor in the production of the crop. For example, in 1935, Boewe (4) reported that considerable damage was caused to the soybean crop in southern Illinois by downy mildew, the fungus destroying on an average some 10 per cent of the leaf area and sometimes attacking every plant in a field. In Canadian pathological literature on the soybean, references of a similar nature may be found as, for example, one by the present authors (18) who, in 1946, expressed the opinion that the extreme susceptibility of Capital to downy mildew might affect its survival as a commercially acceptable variety. Although these references—and others which could be quoted—imply that more or less serious losses may be caused by the disease, they lack definite and substantiating evidence in this regard. The present investigations are probably the most comprehensive that have been carried out in connection with the disease since the pioneer work of Lehman and Wolf (20), and they supply the most definite information to date as to the role the pathogen really plays in the production of soybeans at least under Ontario conditions.

From the standpoint of practical agriculture, a most important criterion for determining the significance of a disease is the effect of the latter on yield. Adjudged on this basis, the results presented above strongly suggest that the downy mildew is not—nor is likely to become—a significant limiting factor in the production of soybeans in the province of Ontario. When both treated and untreated, oospore-encrusted seed was planted in a series of 5 tests over a 3-year period, the yield from treated seed was only in one instance significantly greater than that from untreated seed, despite the fact that in all tests the effect of seed treatment was virtually to eliminate systemically infected plants. The significant increase in yield obtained in the one case is of negligible practical importance. It only serves to emphasize the fact that the mildew-infected fraction of a given seed-lot would have to be very high before a successful control measure, such as seed treatment, would be likely to increase yield. In the seasonal examination of soybean seed over a period of 10 years, the oospore-encrusted seeds in any given seed-lot were found never to exceed 11 per cent. The results

of the present investigations show further that there was no significant difference between yields obtained on the one hand from oospore-encrusted seed and on the other from healthy seed of the same variety, treated and untreated, respectively.

In 1946, Jones and Torrie (17) found in their studies of downy mildew of soybeans that varieties differed in the kind and the abundance of foliar lesions which developed in the field during the summer and that foliar infection apparently had no effect on yield of seed. The present authors also have found lack of correlation between foliar infection and yield. In Table 2 it may be noted that non-treated, oospore-encrusted seed of the variety A. K. Harrow gave rise in outdoor plots in 1946 and 1948 to 5.4 and 7.0 per cent systemically infected plants, respectively. These percentages represent actual totals of 30 and 187 plants, the majority of which in the two years became centres from which inoculum spread and infected varying numbers of neighbouring healthy plants. Thus, the plants showing heavy foliar infection could, in the aggregate, be numbered in the hundreds. Yet, the yield from the plots containing all these infected plants was not significantly different from that of plots in which the plants, having originated from treated seed, were relatively free from either systemic or foliar mildew infection.

From the foregoing it is apparent that the present studies have revealed several interesting anomalies. The disease can be almost completely controlled by seed treatment yet, as a result, yield is not increased; nor is yield significantly adversely affected either by appreciable percentages of systemically infected plants or by heavy foliar infection. The most difficult anomaly to explain was encountered in the experiment in which healthy seed of the varieties Harman and A. K. Harrow were planted in contact with healthy and with oospore-bearing pods of the two varieties. It will be recalled that systemic infection occurred only when healthy seed was planted in contact with the infected pods, and even then the percentage of infected plants was extremely low (Harman 1.6, A. K. Harrow 0.3 per cent). However, for both varieties, the yield from healthy seed planted in contact with the infected pods was significantly less than that from the healthy-seed, healthy-pod planting combination. Such results would almost suggest that a considerable number of plants may have been infected without showing visible symptoms. This is only one of many different points in respect to this disease which remain to be determined.

SUMMARY

Downy mildew of soybeans, caused by *Peronospora manshurica* (Naoumoff) Sydow, was first discovered in Manchuria in 1921 and has since become almost coextensive with the cultivation of the host.

Soybean plants exhibit two types of infection, systemic and local. Systemic infection originates from infected seed, the mycelium and especially the oospores of the pathogen being borne primarily on or within the seed coat. Evidence based on microscopic examination of plant tissues and on visual progression of symptoms indicates that the growth of the fungus, though not immediately concurrent with that of the plant, finally pervades all parts of the latter. Systemically infected plants produce an

abundance of conidia which, when blown by the wind or transported otherwise to surrounding healthy plants, induce in the latter the local type of infection.

Some systemically infected plants die prematurely, others exhibit a spindly type of growth and mature few seeds, while still others, though exhibiting foliar symptoms, grow vigorously and produce a quota of infected seed which almost equals that of healthy plants.

The highest incidence of systemically infected plants encountered in greenhouse plantings of non-treated, oospore-encrusted seed was 37.0 per cent; in field plantings 12.9 per cent.

From greenhouse plantings of oospore-encrusted seed of the varieties Harman and A. K. Harrow, stored over winter on the one hand in a heated laboratory and on the other in an unheated granary, evidence has been adduced which indicates that not only may variety and conditions of storage be inter-related but that each of these factors may also be correlated with the incidence of systemically infected plants.

From 1946 to 1948, inclusive, oospore-encrusted seed of the varieties Harman and A. K. Harrow was treated with Spergon and Fermate and planted together with untreated seed of the two varieties in outdoor plots in a randomized, 5-replicate design. Treatment with the protectants increased emergence in 4 of the 5 trials, virtually eliminated the incidence of systemically infected plants in all 5 trials, but in only one of the 5 trials resulted in a significant increase in yield.

In general, the results suggest that the disease is not—nor is likely to become—a significant limiting factor in the production of soybeans in southwestern Ontario.

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A HISTOLOGICAL STUDY OF ICE FORMATION IN FROZEN VEGETABLE TISSUES[1]

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PART I. A Review of the Changes which Occur in Freezing

INTRODUCTION

Although a great deal of experimental work has been done in the frozen food field, there still remains some uncertainty as to which are the best methods of processing and freezing. One need only consult a number of publications recommending methods of blanching, preparing and freezing to notice the variation in recommendations. There has also been some controversy over the merits of quick and slow freezing of foods. With this in mind, it was decided that a study of the changes which occur in freezing followed by a number of experiments using different rates of freezing with several vegetables might throw some new light on the subject.

Slow versus Quick Freezing

The advantages of quick freezing were summarized by Tressler and Evers (25). They stated that: "It is a generally accepted fact that quick freezing is superior to slow freezing", and mentioned the following as the main advantages of quick freezing:

"1. The ice crystals formed are much smaller and therefore cause less damage to the cells.

"2. The freezing period being much shorter, less time is allowed for the diffusion of salts and the separation of water in form of ice.

"3. The product is quickly cooled below the temperature at which bacterial, mould and yeast growth occurs, thus preventing decomposition during freezing."

MacArthur (14), Eickelberg (6), Joslyn and Marsh (12), and others support the views stated above.

On the other hand, there are a number of reports which claim that the slower rates of freezing are not necessarily injurious to the frozen product.

Diehl and Berry (4) stated: "It would seem that very rapid freezing is not essential to quality retention in frozen pack peas and that temperatures near 0° F. are satisfactory providing reasonably rapid heat transfer is obtained in cooling the product and finally freezing it. Experiments with other vegetables indicate that this is also true in a general way for asparagus, lima beans, corn, spinach, cauliflower and beans, although minor modifications exist for some products."

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Lee, Gortner and Whitcombe (13) found, on final analysis, that there were no significant differences in taste and texture of vegetables which had been frozen at rates ranging from very rapid, by means of liquid air, to very slow, in an insulated box.

Plagge (18) found, as a result of a review of literature, that "in certain instances, good or better products have been reported by slow or moderate freezing and also . . . . slow freezing such as practised in many locker storages gives satisfactory results."

Other workers, including Caldwell, Lutz, Moon and Myers (1), and Woodroof (26) support these views. In a later work, Woodroof (27) concluded that the temperature requirements for successful freezing were quick freezing, rapid hardening, and constant storage, all of which were successfully accomplished at 0° F.

Changes Occurring During Freezing

Freezing of foods is practised to retain as far as possible the properties of the fresh vegetable, fruit or other product. It is well known, however, that certain irreversible physical and chemical changes take place during the freezing and thawing processes. It is also recognized that the appearance and texture of frozen foods differ from the fresh. After thawing, the product is usually limp, due to collapse of tissues and a certain amount of "drip" liquid is lost. Succulent tissues are inclined to lose more liquid than tissues containing starch cells. Starch, evidently, acts as an additional support to the cells.

Woodroof (26) summarized the various theories offered in explanation of these changes. He stated that flabbiness was the result of the breaking down of the contents of the cells which formerly gave support to their walls. The loss of plant juices was not due to the rupture of the cell walls but to the irreversible precipitation of cell contents liberating "bound water" as well as water which was previously present in the vacuole, the water not being re-absorbed on thawing. Woodroof (26) found that most of the water lost through cell leakage was very similar to that lost through leakage caused by cooking, lost by placing the tissue in saturated salt or sugar solution, or lost when undergoing desiccation. He also noted that tissues having very thick cell walls did not show as marked changes as those with thin cell walls, owing to the fact that support of the tissues was largely maintained by the cell walls.

Several theories have been presented in explanation of the changes that occur in plant tissues on freezing and thawing. Woodroof (27) agreed with the theory that injury was caused by puncturing of the cell walls by ice crystals. He wrote: "Ice crystals puncture cell walls. This is easily done since the cell walls are thin and offer little physical resistance to the growth of ice crystals, and crystallization is too rapid for much water to be drawn into intercellular spaces before freezing."

Duhamel and Buffon (5), in 1737, postulated that ice formation inside a cell would rupture the cell wall by expansion. Geoppert (7), however, proved by direct observation that cell walls were not ruptured in the above manner.

Wiegand (28), who investigated ice formation in tree buds during winter, found that ice formed a continuous layer always in the mesophyll of the leaf or scale but was never found between scales. He found that, on either side of the ice sheet, the cells were in a state of collapse and very often occupied less space than the leaf itself. He found no injury in buds even after rapid thawing at 21° C.

Sommerville in England and Sachs in Germany thought that the injury was due to too rapid thawing. Sachs first brought out this idea in 1860 and the idea was revived in 1933 by Iljin (10). Iljin proposed that death occurred as a result of subsequent thawing of the tissues and not at the time of ice formation. He pointed out that some plant tissues which can withstand freezing were killed by rapid thawing. Death was apparently due to various types of mechanical disturbances attendant upon the entry of water into the cells upon thawing.

Iljin's observations (9, 11), as quoted by Scarth (19), showed that the main damage in thawing was caused by the rapid stretching in deplasmolysis and rupture of the protoplasm.

Maximov (15) and Chandler (2), working independently, came to the conclusion that injury was not due to cold itself but to ice formation.

According to Maximov (15), the ready loss of sap by frost-killed organs for a long time was explained as due to the fact that freezing water, in expanding, ruptured the cell walls. He believed this supposition to be false. He showed that water contained in cell walls was frozen first and ice was formed not in the cells but in intercellular spaces. He pointed out that cell walls in frost-killed plants remained uninjured. Death was due not to rupture of cell walls but to changes in the protoplasmic membrane, primarily to its coagulation. This is the result of the drawing out of water from cells by ice formation in the intercellular spaces. Cell sap becomes more concentrated and protoplasm becomes exposed to the pressure of growing ice crystals. This results in an irreversible coagulation of the colloidal substances of the protoplasm. After thawing, permeability is lost because protoplasm is dead.

Schander and Schaffnit (21), as well as Iljin (9), said that ice crystals formed and grew in the intercellular spaces and neither pressed upon the cells nor even touched the protoplasm.

Scarth and Levitt (20) noted that ice formation within the cell did sometimes occur and that it was confined to the vacuole. The results of such ice formation were nearly always fatal. The mechanism of such injury may be, as suggested by Iljin, the compression of the protoplasm between the freezing and expanding sap on the inside and the cell wall or rigid hull of ice on the outside; or it may also be laceration of the vacuolar membrane and other structures by the ice crystals.

Chandler (2), in summing up the ice-formation theory, stated: "There can be no injury by cold without ice formation."

Meyer and Anderson (16), in reviewing the killing of plant tissues by ice formation, came to the conclusion that the factors which determine whether freezing will occur within the cells or between them are not well understood. Freezing in the intercellular spaces is apparently much less often injurious to cells than freezing within the cells.

Harvey (8) was of the opinion that withdrawal of water from the cells, due to ice formation in the intercellular spaces, resulted in an increase in the concentration of electrolytes in the cell sap which may have a "salting out" or other destructive effect on the protoplasmic proteins.

Stuckey and Curties (24) in 1938, and Siminovitch and Scarth (22) in the same year, reported independently that in intracellular freezing, ice crystals appeared first in the cytoplasm and then in the vacuole. The visible effects of frost injury as observed after thawing were reported by Scarth (19) to be a contracted and coagulated protoplasm with frequently a frothy structure. Except for the degree of contraction this appearance was not very distinctive from what is produced by many other killing agents.

Stiles (23) and others believed that ice crystals formed within the cells resulted in compression or laceration of the protoplasm and plasmodesmata or other destructive effects.

Most investigators (2, 3, 12, 15, 16) favour the concept that injury to protoplasm during freezing is fundamentally due to mechanical effects of ice formation either within or between the cells rather than to dehydration or to chemical effects. The ultimate effect of such mechanical disturbances is presumably the disruption of the delicate organization of the protoplasm.

Physical and Chemical Changes

Joslyn and Marsh (12) found that the physical changes that occur during freezing storage depended chiefly upon ice formation and osmotic action; they involve changes in volume, drained weight and texture. These physical changes occur primarily during freezing and during thawing out generally not during freezing storage, especially if a constant storage temperature is maintained.

The chemical changes that occur during preparation, freezing and thawing involve changes in composition such as hydrolysis of pectin and sucrose by hydrolytic enzymes which are present; changes in colour and flavour due to oxidation, largely arising through the activity of oxidizing enzymes; and also changes in flavour due to anaerobic respiration and other causes. These changes are especially marked during the thawing period, owing to injury to the tissues during freezing.

PART II. Experiments with Beet Petioles, Asparagus, Beans and Peas

As indicated in the first part of this paper, the vegetables used in these experiments consisted of beet petioles, asparagus, beans and peas. Three rates of freezing were used: (1) Frozen at "Plates" (frozen on cold plates at −39° F. and kept there until microscopic examination). (2) Frozen "Plates to Room" (frozen at −39° F. and removed to holding room temperatures at 0° F. to 5° F. after 24 hours). (3) Frozen at "Room" (frozen at holding room temperature of 0° F. to 5° F. and maintained there).

The actual time of complete freezing was measured by inserting a low-temperature thermometer in the centre of individual packages and freezing these in the normal manner. Readings were taken at frequent regular intervals. Some of the results are shown graphically in Figure 1.

HISTOLOGICAL TECHNIQUE

It was found that the most satisfactory way of obtaining sections of the frozen materials was by free-hand, using new razor blades. The sections were made rapidly and good specimens were placed on glass slides. Several mounting liquids were tried; among these were F.A.A. killing solution oil and glycerine. None was found to be suitable. In this work the important factor was to observe reflected light from transparent ice crystals but when any of the above-mentioned liquids were placed on the ice-containing sections, the edges of the crystals became almost invisible thereby defeating the purpose of the microscopic examination. Consequently the sections were mounted dry. The most satisfactory method of mounting consisted of applying a very slight amount of heat on the lower part of the slide by means of a finger tip. Care had to be taken to not ruin the sections with too much heat. From two to six sections were placed on each slide and these were examined immediately. Fresh and thawed samples were also examined. Photomicrographs were taken of representative samples.

EXPERIMENTAL RESULTS

The actual freezing point, or the point where most of the water changes to ice, varied with different vegetables and with different rates of freezing as shown in Figure 1 and Table 1. Beet tops frozen at "Room" remained at approximately 28.6° F. for about four hours, while beets frozen at "Plates" remained at 26.6° F. for 1½ hours. Beans frozen at "Plates", on the other hand, reached 21° F. and maintained that temperature for 1½ hours. Beans frozen at "Room" took four hours and twenty minutes to pass the actual point of freezing. The rates for peas were similar to those for beans.

TABLE 1.—RECORDED FREEZING RATES OF BEANS, BEET PETIOLES AND PEAS

Material and freezing condition	Time from start to 0° F.	Time of actual freezing	Temperature reached	Total time
Beet at 0° F. to 5° F.	8 hr. 45 min.	4 hr. 20 min.	0° F.	9 hr. 0 min.
Beet at − 39° F.	3 hr. 20 min.	1 hr. 40 min.	−22.9° F.	4 hr. 55 min.
Bean at 0° F. to 5° F.	8 hr. 25 min.	4 hr. 10 min.	0° F.	9 hr. 25 min.
Bean at − 39° F.	3 hr. 10 min.	1 hr. 40 min.	−34.6° F.	6 hr. 0 min.
Pea at − 39° F.	3 hr. 25 min.	1 hr. 40 min.	−29.2° F.	5 hr. 25 min.

The materials frozen at "Plates" took between 190 and 200 minutes to reach 0° F., while the vegetables frozen at "Room" took approximately nine hours to reach 0° F.

Examination of Beet Tissues

Petioles of Detroit Dark Red beets, grown in the greenhouse, were examined microscopically. Figures 2 and 3 illustrate the structures found in fresh tissues. They show the presence of vascular bundles, with xylem and phloem structures such as spiral vessels, sieve tubes and companion cells as well as undifferentiated parenchyma cells.

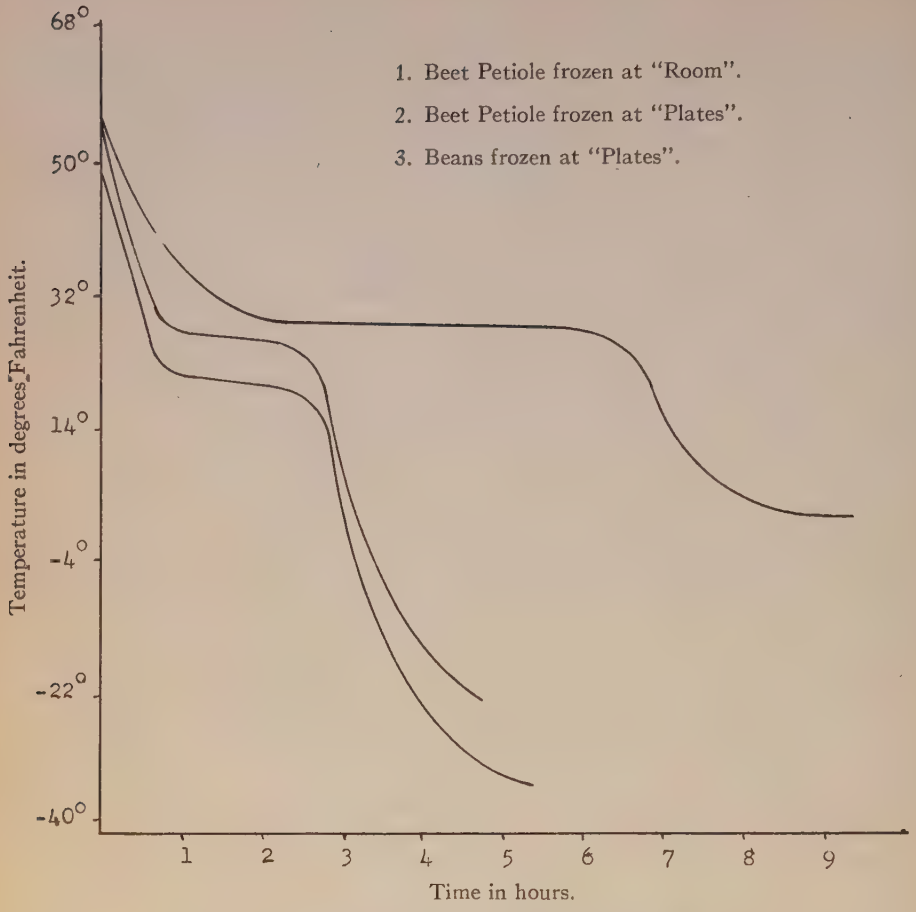

FIGURE 1. Rates of freezing of bean and beet tissues.

Petioles were frozen at the three rates. Those frozen at "Plates" contained a large amount of intracellular ice as well as some extracellular ice. Vascular bundles were somewhat damaged by ice within them. Some parenchyma cells appeared plasmolyzed but most were normal. Cell walls did not appear to be damaged. Figures 4 and 5 illustrate these points. Tissues frozen at "Plates" and then thawed showed ruptures in the vascular bundles. Parenchyma cell walls were not damaged but the cells themselves were somewhat disrupted. Ice damage in this parenchyma tissue was confined to the proximity of vascular bundles, and in some cases near the epidermal layer. Ice damage is well illustrated in Figures 6 and 7.

Beet petioles frozen at "Plates to Room" indicated that ice formation is similar to that in the same material frozen at "Plate", viz., in and around vascular bundles as well as within and outside of parenchyma cells. Figure 8 shows ice formation inside a vascular bundle and intracellularly in the parenchyma cells. Thawed samples of this material showed little or no damage in some instances but on the average the appearance of the tissues was similar to that of beet petioles frozen at "Plates". Figures 9 and 10 are typical examples.

The attached page of illustrations (Figures 2–9) was unfortunately omitted from the article "A histological study of ice formation in frozen vegetable tissues", by A. R. Maurer and H. R. Murray, published in the December, 1951 issue of *Scientific Agriculture* (Vol. 31, No. 12). Please insert this page between pages 524 and 525.

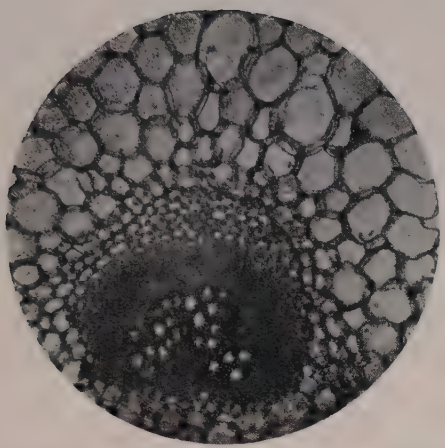

FIGURE 2. Cross section of fresh beet petiole showing parenchyma cells and vascular bundle. X100.

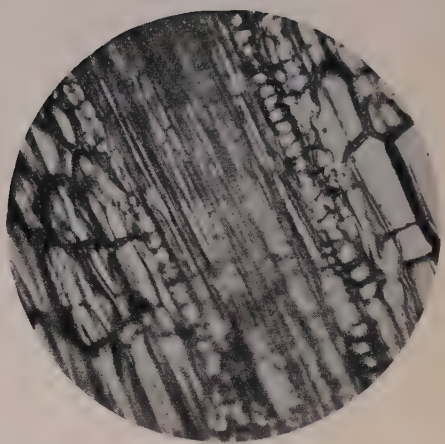

FIGURE 3. Longitudinal section of fresh beet petiole showing parenchyma cells and vascular bundle. X100.

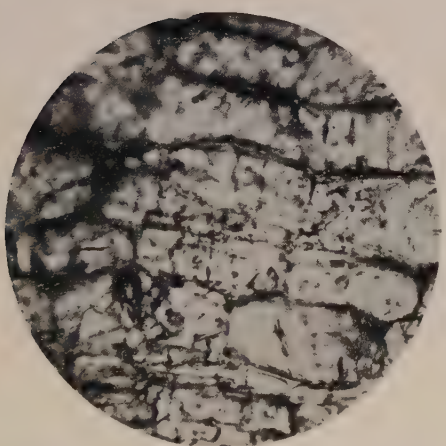

FIGURE 4. Longitudinal section of beet petiole frozen at "Plates" showing parenchyma cells affected by ice. X100.

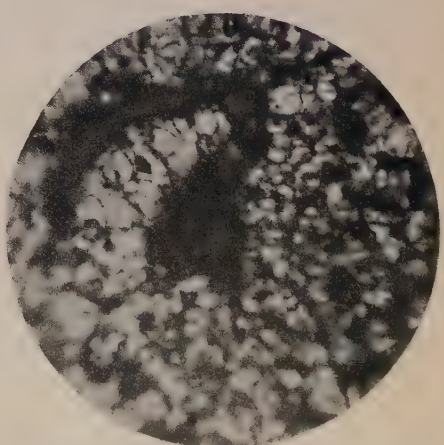

FIGURE 5. Cross section of beet petiole frozen at "Plates" showing vascular bundle with internal ice and parenchyma cells affected by ice. X100.

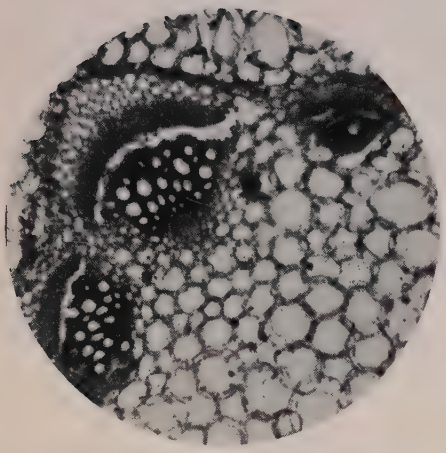

FIGURE 6. Cross section of beet petiole frozen at "Plates" and thawed showing ruptured vascular bundle. X100.

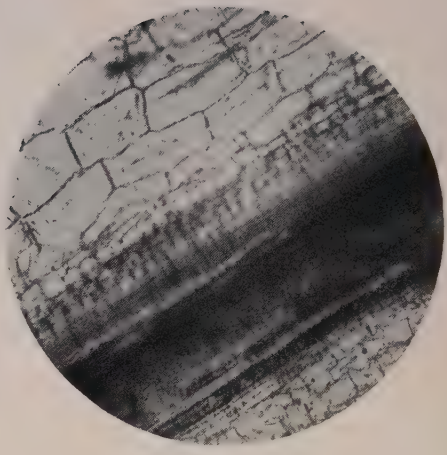

FIGURE 7. Longitudinal section of beet petiole frozen at "Plates" and thawed showing vascular bundle and parenchyma cells. X100.

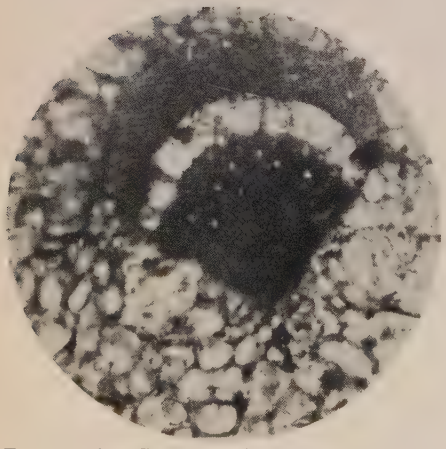

FIGURE 8. Cross section of beet petiole frozen at "Plates" showing a vascular bundle containing a wide band of ice. X100.

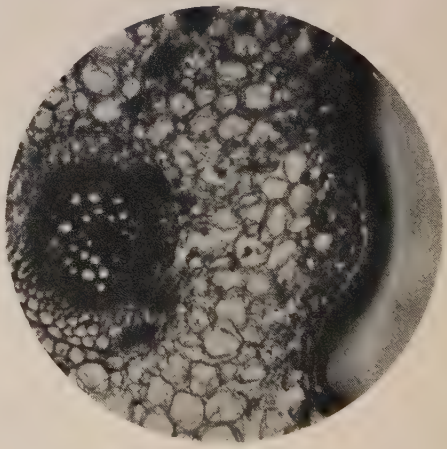

FIGURE 9. Cross section of beet petiole frozen at "Plates to Room" and thawed showing disruption in vascular bundle and parenchyma cells. X100.

Beet tops frozen at "Room" had a different appearance and texture. Ice crystals could be detected with the naked eye. Microscopic examination indicated that ice was formed within the vascular bundles and parenchyma cells as well as outside the cell walls. Figure 11 shows a vascular bundle with a wide band of ice and parenchyma cells which appear to be plasmolyzed and which contain some ice crystals. The longitudinal section in Figure 12 shows much ice formation in a vascular bundle and what appears to be disruption of cells. The thawed samples showed tears in vascular bundles and disruption of cells in the vicinity of these bundles. This is illustrated in Figure 13.

Examination of Asparagus Tissues

Asparagus stalks frozen at "Plates" showed the basic structures of a monocotyledonous stem. Vascular bundles were massed beneath the epidermal layers and interspersed throughout the undifferentiated parenchyma in the centre of the stem. These bundles and the undifferentiated cells were well defined. Ice formation was particularly heavy within the bundles, especially in the larger vessels as can be seen in Figure 14. Parenchyma cells showed undamaged cell walls as well as intra- and extracellular ice. After thawing it was apparent that ice damage was confined to vascular bundles and their immediate vicinity. This is illustrated in Figure 15. Ruptures in the bundles indicate that the ice formation was intracellular while damage to the parenchyma tissues would indicate that ice formed between cells. There was little damage to cell walls.

Asparagus tissues frozen at "Plates to Room" appeared very similar to those frozen at "Plates". There may have been a slightly greater amount of damage to vascular bundles while parenchyma cells had more extracellular ice. Otherwise there was little difference. Thawed samples indicated, as before, that these tissues were similar to those frozen at "Plates".

Asparagus stalks frozen at "Room" showed larger ice formation which was visible to the naked eye. The heavier formation was particularly noticeable near the epidermis, i.e., in the region of the massed vascular bundles. Both intra- and extracellular ice was found in the parenchyma tissue. Ice between cells was more noticeable than in similar tissues frozen at the faster rates, as can be seen in Figure 16. Some parenchyma cells appeared coagulated, perhaps as the result of withdrawal of water on freezing. The thawed material showed that ice damage was considerable. Ruptures were not confined to vascular bundles but were also found between parenchyma cells close to bundles as illustated in Figure 17. Otherwise there was little difference from other thawed samples frozen at faster rates.

Examination of Bean Tissues

Microscopic examination of fresh bean tissues showed several distinct cellular layers in the pod—the exocarp made of a layer of small thick-walled cells and several layers of slightly larger cells, the mesocarp, next to the exocarp, consisting of larger thin-walled cells which appear to be undifferentiated, and the endocarp, similar in appearance to the exocarp, forming the inner wall of the ripened bean pod. However, at the immature stage,

an area of undifferentiated cells is found next to the endocarp and throughout the pod. Several parts of the immature seed, viz., the cotyledons, seed coat and, in some cases, the young embryo were also present. Storage cells, containing grains of starch and protein granules were much in evidence in the cotyledon tissues.

Ice formation was observed in all the cells of beans frozen at "Plates". The mesocarp seemed to be particularly affected. Ice was present in some parts of the young seed, the amount depending on the maturity of the seed. There was less ice where food storage cells were in evidence. The mesocarp cells showed both intra- and extracellular crystals and disruption while the heavier-walled exocarp and endocarp tissues were not greatly affected. On thawing, the main damage appeared in the regions where ice structures had been noted.

Examination of beans frozen at "Plates to Room" showed that slight differences in ice structure can occur as a result of holding temperatures. Sections of bean tissues showed marked ice formation in the mesocarp. Greater disruption of tissues was apparent in some instances. The cells of the endocarp appeared to have been pressed together by the effect of ice formation in neighbouring tissues. Cross sections of the immature seed showed extracellular ice around starch and protein storage cells and intracellular ice in the other tissues in this region. As previously found, after thawing, the main ice damage appeared in the regions of greatest ice formation.

Visual observation of beans frozen at "Room" showed that ice structures were particularly heavy in the mesocarp. Under the microscope these samples showed greater ice formation than was found with the other rates. In some cases, ice crystals were not confined to the larger thin-walled cells but were found under the first layer of exocarpic cells. Most regions appeared somewhat disrupted by the formation of ice. After thawing, damage was greater, these tissues were found to have more damage in the mesocarp but were, in all other respects, quite similar to beans processed at quicker rates.

Examination of Pea Tissues

Peas were treated in a manner similar to the other vegetables already mentioned. Cross-sections of pea seeds did not show very much more than a multitude of starch storage cells and other cellular tissues.

Microscopic examination of peas did not show a great deal of difference between the various treatments. Ice formed in the suture between the two cotyledons as well as intra- and extracellularly in the tissues. Slower rates of freezing resulted in larger ice crystals but after thawing no real differences could be noted. In all cases, it was found that starch storage cells tended to separate from each other after thawing, indicating that the tissues had been disrupted in some way by ice formation.

DISCUSSION

It has been pointed out that workers do not agree on the merits of quick and slower freezing. Tressler and Evers (25), for instance, are strong advocates of rapid freezing. Lately, however, there has been a trend towards slower processing. Phillips (17) explains that the reason for this

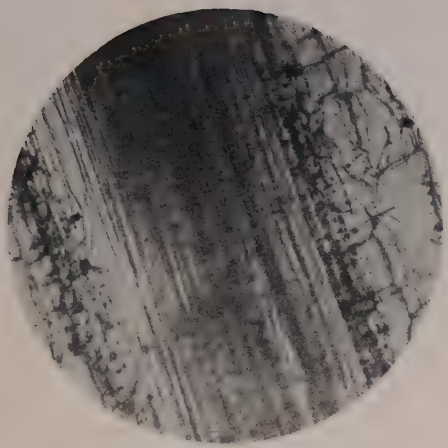

FIGURE 10. Longitudinal section of beet petiole frozen at "Plates to Room" and thawed showing vascular bundle and parenchyma cells. X100.

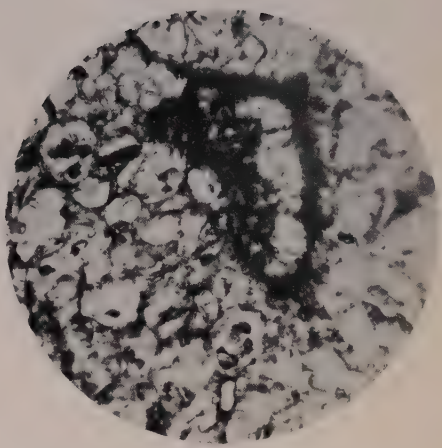

FIGURE 11. Cross section of beet petiole frozen at "Room" showing vascular bundle and parenchyma cells with ice structures. X100.

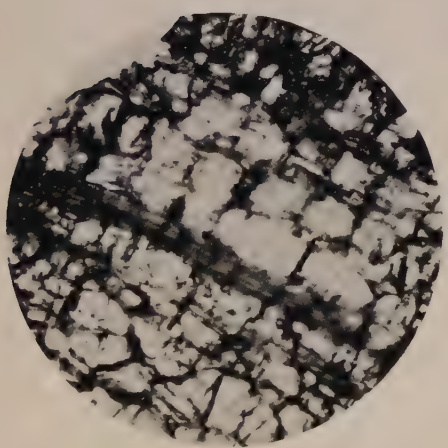

FIGURE 12. Longitudinal section of beet petiole frozen at "Room" showing parenchyma cells with ice structures. X100.

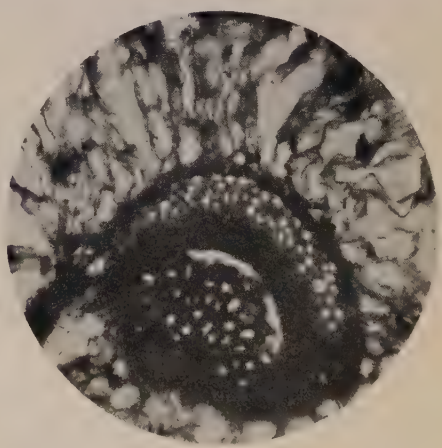

FIGURE 13. Cross section of beet petiole frozen at "Room" and thawed showing slight damage to vascular bundle and damage to parenchyma cells. X100.

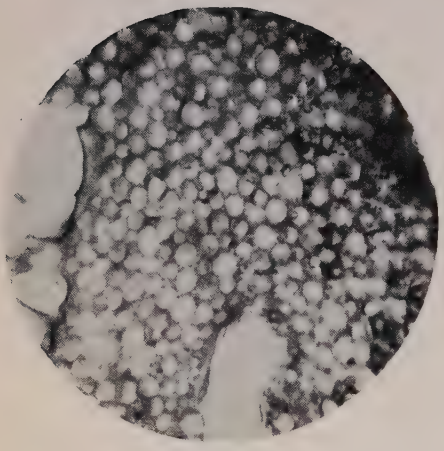

FIGURE 14. Cross section of asparagus frozen at "Plates" showing vascular bundle containing much ice and parenchyma cells apparently undamaged. X100.

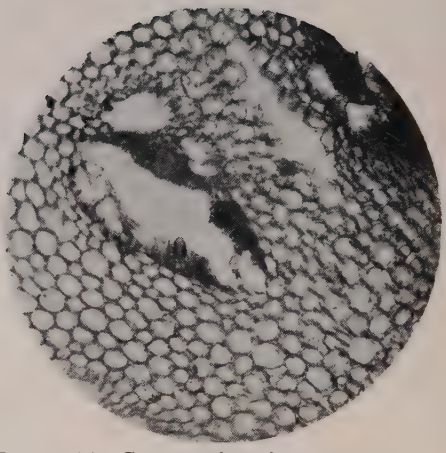

FIGURE 15. Cross section of asparagus frozen at "Plates" and thawed showing damage to vascular bundle and tear in parenchyma tissue. X100.

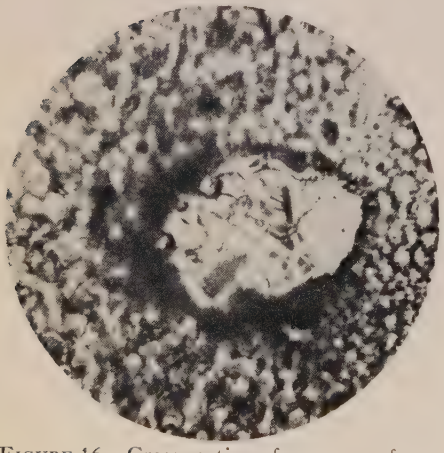

FIGURE 16. Cross section of asparagus frozen at "Room" showing ice formation in vascular bundle. X100.

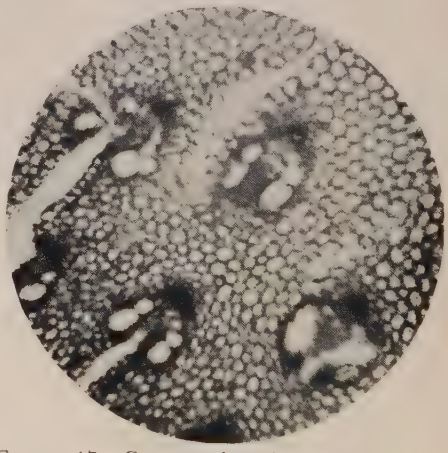

FIGURE 17. Cross section of asparagus frozen at "Room" and thawed showing damage to tissues. X100.

trend is not that quick freezing is, in any way, detrimental to the quality of frozen foods but that slower freezing does not result in products which are inferior. His most rapid freezing was in 2 hours, his slowest in 10.5 hours.

Most work in this line, to date, has been based on results obtained by inspection of thawed products. This paper differs in that examination of tissues was made while the tissues were in the original frozen state thereby removing the possibility of change in ice structure due to a rise in temperature.

The experimental results indicate that size of ice crystals is directly dependent on the rate of freezing. Minute crystals are formed at quick rates and larger crystals result as the rate is decreased. It has also been shown that the amount of extracellular ice is greater in products which are slow-frozen. Intracellular ice was found at all rates used in these experiments but was more noticeable in the fast frozen vegetables. It was found that undifferentiated parenchyma cells of asparagus and beets were not injured greatly by any of the rates of freezing. In the case of beans, however, it was the layer of undifferentiated cells forming the mesocarp which was harmed most, especially by the slower rates.

Vascular bundles in asparagus stalks and beet petioles were the first tissues to be affected by ice formation. It was noticed that in practically all cases ruptures occurred through vascular bundles. In many instances the parenchyma tissues in the immediate vicinity of these bundles were injured by the formation of large ice crystals. These crystals were formed extracellularly and seemed to push the cells apart. Similar ruptures were not found in other regions of parenchyma cells. Although this type of injury was found in asparagus and beet tissues frozen at all rates, the damage was somewhat greater at the slower speed. Ice formation in and about vascular bundles is probably due to the fact that these conducting vessels have a much greater amount of "free" water and that this water freezes first. It is only natural that this free water should freeze before the water present in other cells because of its lesser osmotic concentration. The larger vessel in vascular bundles would also serve as "gathering points" to which water from neighbouring cells would diffuse as ice formation took place. This movement of water out of adjoining cells would take place until the cells themselves reached the freezing point. In this way slow-frozen products would have a chance to accumulate more water in the vascular bundle area and, therefore, would have larger ice crystals than their fast-frozen counterparts. In a similar manner extracellular ice formation would be greater in the undifferentiated cellular areas in slow-frozen tissues. In these experiments, however, it was found that extracellular ice in parenchyma tissues was not of sufficient magnitude to cause complete distruption. This is illustrated in the photomicrographs of thawed tissues which show the cell walls to be intact in practically every case.

Frozen beans, having no structures corresponding to the vascular bundles of beets and asparagus, formed the most ice in the mesocarp tissues. This means that ice damage is greatest in the layer of cells immediately below the exocarp of the bean pod. Some disruption also occurred in the area of undifferentiated parenchyma cells next to the endocarp.

As before, ice injury was more noticeable in the slow-frozen material. In the seed of beans, it was found that the main ice formation occurred in the suture between the two cotyledons. Ice crystals were also found intracellularly and extracellularly in the cotyledons and other parts of the immature embryo. It was noticed that starch and protein storage cells were not particularly injured by ice formation. This is most likely due to their very low content of "free" water and their high osmotic pressure. Food storage cells, containing starches and proteins, have the greater part of their water in the "bound" form and are, therefore, less likely to suffer from ice injury than parenchyma cells which contain so much "free" water.

It was noticed that ice formation in peas was similar to that found in bean seeds. It was also noted that the extent of damage did not only depend on the rate of freezing but also on the maturity of the seed. The more mature seeds contained a greater proportion of food storage cells and these were not harmed to the same extent as adjoining ordinary or undifferentiated cells.

In every case it was noticed that a large amount of "drip" liquid was lost by the thawed materials. This liquid is probably the result of melting extracellular ice and it contains cellular constituents which are allowed to escape through the cell walls, the latter having lost their permeability due to the killing effect of intracellular ice.

It is the opinion of the author that damage to tissues and death of cells is caused by the mechanical action of ice formation; ice being detrimental to the cellular structures and also withdrawing part of their water content, thereby upsetting the internal balance in these tissues.

From subsequent examination of all these materials in the thawed condition, it is concluded that the only real advantage of quick-freezing is in saving time in commercial operations. Otherwise it would not seem practical to use more costly quick-freezing apparatus where a cheaper, slower freezing system would do just as well. Examination of the materials, prepared under the conditions of these experiments, indicates that although there is a difference in ice injury at the slower rates of freezing, this difference can be assumed to be insignificant as far as the final cooked product is concerned. Representative samples of all these products were cooked in the laboratory and when submitted to a tasting test, no real differences were found.

SUMMARY

When frozen foods are subjected to different rates of freezing there is a direct relationship between size of ice crystals and rate of freezing; the slower the speed, the larger the crystals.

Ice crystals occur intracellularly and extracellularly, there being a greater proportion of extracellular ice in products frozen at slower rates.

Ice is first formed in and around the water conducting vessels of vascular bundles due to the presence of "free" water in these tissues. Ice crystals in these areas cause ruptures not only in the vascular bundles but also in the neighbouring parenchyma cells. Walls of other parenchyma cells are not harmed by ice.

Injury to tissues is caused by the mechanical action of ice formation both intracellularly and extracellularly.

The loss of liquid on thawing is due to the melting of ice crystals formed outside cell walls which have been rendered permeable by the action of ice in killing the tissues.

The results of these experiments would indicate that asparagus, beans, beet-tops and peas frozen at the slow rate used in this project are not, on final analysis, inferior in quality to similar vegetables frozen at the more rapid rates.

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BACTERIAL SPOILAGE IN PROCESS CHEESE[1]

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In a previous paper (1) *Clostridium sporogenes* was reported to be the main organism responsible for spoilage in process cheese. Earlier studies also indicated that the main source of infection was skim milk powder, one of the ingredients of process cheese. While Cheddar cheese, the basic constituent of process cheese, was also suspected as a source of infection, this was not confirmed in earlier work. It is now quite evident that Cheddar cheese as well as skim milk powder may be a major source of spoilage organisms, as defective samples of process cheese have recently been submitted to the laboratory which contained no skim milk powder. That spore-forming gas-producing organisms other than *Clostridium sporogenes* may be implicated in spoilage has been shown from an examination of spoiled process cheese, Cheddar cheese and Blue cheese samples submitted from other troubled processing plants. Studies have therefore been continued to determine (*a*) source and identity of clostridia associated with spoilage; (*b*) the incidence of clostridia in skim milk powder and raw milk Cheddar cheese; (*c*) reproducibility of the defect in experimental process cheese with various isolated clostridia; and (*d*) commercial methods of control.

SOURCES AND IDENTITY OF CLOSTRIDIA ASSOCIATED WITH SPOILAGE

For the isolation of clostridia, a 5 gm. portion of the defective or suspected cheese was weighed into a small sterile mortar. To this was added 5 to 10 ml. of sterile 1.0 per cent sodium citrate and the sample thoroughly mixed with a sterile pestle. This emulsion was returned to the 45 ml. dilution blank and the mortar and pestle rinsed once with the solution from the dilution blank. The 0.1 dilution was shaken 50 times by hand and a 1 ml. aliquot transferred to a 99 ml. 1.0 per cent sodium citrate dilution blank thus giving a 0.001 dilution. Melted, semi-solid, "Difco" brain liver heart medium + 0.75 per cent agar, dispensed in tubes plugged at the bottom with a rubber stopper, was inoculated in duplicate with 0.1, 0.01 and 0.001 gm. from the appropriate dilutions to give well isolated colonies. The tubes were then heated to 80° C. for 20 minutes and incubated for 48 hours at 37° C.

Examination showed aerobic surface growth in a high percentage of the tubes. From the tubes showing gas and a characteristic foul odour, anaerobic spore-forming bacteria were isolated in all instances by removing the rubber stopper and transferring the lower one-third of the medium to a sterile Petri dish. A loopful of material was then transferred to a tube of melted and tempered semi-solid medium and serial dilutions prepared in identical tubes by transferring 1 ml. aliquots. Tubes were incubated for

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[3] Assistant Bacteriologist.

48 hours at 37° C. In this way tubes that contained well-isolated colonies were obtained from all samples. Serial dilutions were again prepared as above and isolated colonies picked for further study and identification.

Morphology, gram stain, temperature relations, relations to free oxygen, cultural characteristics in plain and dextrose agar plates and strokes, gelatine plates and stabs and in dextrose and nutrient broths, action on litmus milk, nitrates, coagulated albumin and meat medium, production of indole and hydrogen sulphide, production of acid and gas in dextrose, levulose, galactose, lactose, sucrose, maltose, starch, inulin, glycerol, mannitol, and salicin were determined for the isolated organisms. While minor differences were found, particularly in the sugar fermentations, all the species identified conformed to the general descriptions given in Bergey's Manual (2). Gas-producing clostridia were isolated as follows:

1. Spoiled process cheese:	*Clostridium sporogenes*
	Clostridium pasteurianum
2. Spoiled process Blue cheese:	*Clostridium pasteurianum*
3. Suspected Cheddar cheese:	*Clostridium tertium*
	Clostridium sporogenes
4. Suspected Blue cheese:	*Clostridium perfringens*
	Clostridium sporogenes

INCIDENCE OF CLOSTRIDIA IN SKIM MILK POWDER AND RAW MILK CHEDDAR CHEESE

To determine the incidence of gas-producing clostridia in skim milk powder an additional 100 samples, selected at random from samples submitted from all parts of Canada for grading, were examined by methods similar to those outlined for the detection of clostridia in spoiled and suspected cheese samples, with the exceptions that (*a*) only 0.1 gm. was used and only to inoculate a single tube of medium, and (*b*) two additional media were used, namely, "Difco" cooked meat + 1.0 per cent agar and "Difco" egg meat + 1.0 per cent agar media. Since microscopic examination of material from the bottom of tubes which produced gas showed clostridia in 100 per cent of the tubes when the gas was accompanied by a foul odour and in 70 per cent of the tubes when an odour was absent, the development of gas was regarded as presumptive evidence that gas-producing clostridia were present. The percentage of skim milk powders which showed gas in the different media after the various incubation periods is shown in Table 1.

TABLE 1.—THE PERCENTAGE OF SKIM MILK POWDER SAMPLES SHOWING GAS (PRESUMPTIVE EVIDENCE FOR GAS-PRODUCING CLOSTRIDIA) IN ANAEROBIC MEDIA AFTER 2, 4 AND 7 DAYS' INCUBATION AT 37° C.

"Difco" medium	Per cent showing gas		
	2 days	4 days	7 days
Brain liver heart; cooked meat; egg meat	52	64	68
Cooked meat; egg meat	50	59	63
Cooked meat; brain liver heart	48	57	58
Egg meat; brain liver heart	47	58	61
Cooked meat	43	47	48
Egg meat	38	46	49
Brain liver heart	38	33	38

To determine the incidence of gas-producing clostridia in raw milk Cheddar cheese 51 samples, varying in age from 2 months to 3 years, collected from a commercial cheese warehouse, were examined by methods similar to those previously outlined for skim milk powder. Since microscopic examination of material from the bottom of tubes which produced gas showed clostridia 100 per cent of the time, gas production was regarded as presumptive evidence that gas-producing clostridia were present. The percentage of cheese samples which showed gas in the different media after the various incubation periods is shown in Table 2.

TABLE 2.—THE PERCENTAGE OF RAW MILK CHEDDAR CHEESE SAMPLES SHOWING GAS (PRESUMPTIVE EVIDENCE FOR GAS-PRODUCING CLOSTRIDIA) IN ANAEROBIC MEDIA AFTER 2, 4 AND 7 DAYS' INCUBATION AT 37° C.

"Difco" medium	Per cent showing gas		
	2 days	4 days	7 days
Cooked meat; egg meat; brain liver heart	51	92	94
Cooked meat; egg meat	51	92	94
Cooked meat; brain liver heart	51	92	94
Egg meat; brain liver heart	51	92	94
Cooked meat	49	88	88
Egg meat	49	86	88
Brain liver heart	51	88	90

The results show that, in examining skim milk powder and Cheddar cheese samples, no one medium detected all of the samples which gave presumptive evidence for gas-producing clostridia. The differences between the total per cent of powdered milk samples that showed gas and the per cent that showed gas in any one medium prompted an investigation to determine if the size of the inoculum influenced the occurrence of gas production. Accordingly, 26 of the skim milk powder samples that showed gas in at least 1 medium but not in 3 media, in the above experiment, were examined by methods previously outlined with the exception that 0.5 gm. instead of 0.1 gm. of inoculum was used.

Twenty-five of the samples developed gas in all 3 media when 0.5 gm. of inoculum was used compared with none of the samples when only 0.1 gm. of inoculum was used. This result shows that the size of the inoculum apparently influenced the occurrence of gas, indicating that a minimum number of spores of gas-producing clostridia, which varies with the medium and the organism, is required to initiate growth. Any one of the media would therefore readily detect a heavily contaminated sample of skim milk powder or raw milk Cheddar cheese even when using the higher dilutions as the inoculum would contain the minimum number of spores necessary to obtain a positive presumptive test. Although the test gave presumptive evidence that gas-producing clostridia were present, no indication was given as to whether or not the organisms were capable of causing spoilage in process cheese. To obtain the maximum number of tubes showing gas a minimum incubation period of 4 days at 37° C. is recommended.

It is evident from the results that gas-producing clostridia are widely distributed in raw milk Cheddar cheese and dry skim milk, indicating that these ingredients may on occasion contain the right type of clostridia in sufficient numbers to produce spoilage in process cheese.

REPRODUCTION OF THE DEFECT

In order to provide some information on the ability of isolated clostridia to produce spoilage in experimental process cheese, studies were undertaken to establish the following points: (A) The effect of facultative bacilli, observed in all spoiled process cheese, and readily available nutrients, present in process cheese made with old Cheddar cheese, on the behaviour of different added clostridia in association with a *Bacillus* species and casein digest, respectively; (B) To determine if the number of clostridia and the pH were in some way responsible for the lack of consistent results experienced in both commercial and experimental batches of process cheese; (C) The effect of pH and sodium chloride on the behaviour of clostridia in laboratory media, and (D) The effect of pH and sodium chloride with and without added casein digest on the growth of clostridia in experimental process cheese.

A. Effect of a Bacillus Species and Casein Digest

To study these factors, process cheese was prepared by heating the ingredients—75 per cent Cheddar cheese, 10 per cent skim milk powder, 2 per cent sodium citrate, 13 per cent of water—to a smooth consistency in a double boiler. Inoculum of each clostridium was prepared by mixing the top turbid portion from a vigorously agitated 10-day cooked meat culture with a 10-day brain liver heart culture. Inoculum of a facultative *Bacillus* species isolated from spoiled process cheese was prepared by suspending the growth from a 10-day tryptone glucose agar slant in 10 ml. of 0.9 per cent saline. Twenty per cent casein digest was freshly prepared in distilled water. Sterile 150-ml. beakers were inoculated in duplicate as required with 1.0 ml. of clostridia inoculum, 0.5 ml. of bacillus inoculum and 1.0 ml. of casein digest suspension. A 50 gm. portion of hot process cheese was then weighed into the beakers and mixed with the inoculum, care being taken to avoid the inclusion of air. All beakers were covered with a $\frac{1}{4}$-inch layer of vaspar and incubated for 8 days at 37° C.

Table 3 shows the influence of casein digest and a *Bacillus* sp. on the behaviour of eight gas-producing clostridia in experimental process cheese.

The results show that typical spoilage was inconsistently produced by *Cl. pasteurianum*, *Cl.* sp. (A), which closely resembles *Cl. pasteurianum*, *Cl. sporogenes* and *Cl.* sp. (B), which closely resembles *Cl. sporogenes*. Since *Cl. tertium*, *Cl. perfringens*, *Cl.* sp. (C) and *Cl.* sp. (D) did not produce the defect as shown in Table 3 they were not considered to be responsible for spoilage outbreaks of this nature. However, where spoilage was produced through the various batches of experimental process cheese, it was so inconsistent that no effect could be attributed to either the *Bacillus* sp. or the casein digest although when the defect developed a slightly more pronounced type of spoilage resulted with the use of casein digest.

B. Effect of pH and Load

The lack of consistency, by any of the organisms, in reproducing the defect throughout all of the experimental trials reported in Table 3 indicated that factors other than those under investigation were involved.

TABLE 3.—THE INFLUENCE OF CASEIN DIGEST AND A *Bacillus* SP. ON THE BEHAVIOUR OF 8 SPECIES OF THE GENUS *Clostridium* IN EXPERIMENTAL PROCESS CHEESE HELD 8 DAYS AT 37° C.

Clostridium and source	Process cheese only		Process cheese + cas. dig.		Process cheese + *B.* sp.		Process cheese + cas. dig. + *B.* sp.	
	Duplicate		Duplicate		Duplicate		Duplicate	
Cl. sporogenes—spoiled process cheese	−	−	++++	++++	+++	+++	−	−
Cl. pasteurianum—spoiled process cheese	+++	+++	++++	++++	+++	++++	−	−
Cl. tertium—suspected Cheddar cheese	−	−	−	−	−	−	−	−
Cl. perfringens—suspected Blue cheese	−	−	−	−	−	−	−	−
Cl. sp. (A)—spoiled process no skim milk	++++	+++	++++	++++	+++	+++	−	−
Cl. sp. (B)—Cheddar cheese	++++	+++	−	−	+++	++	−	−
Cl. sp. (C)—Cheddar cheese	−	−	−	−	−	−	−	−
Cl. sp. (D)—skim milk powder	−	−	−	−	−	−	−	−
Control cheese	−	−	−	−	−	−	−	−

++++ Very pronounced gas.
+++ Pronounced gas.
++ Definite gas.
+ Slight gas.
− Absent.

In order to determine if the load of organisms inoculated was in some way responsible for the inconsistent positive results obtained in Table 3, three additional batches of experimental process cheese were prepared and inoculated, by methods identical with those described for the above experiment, with varying numbers of spores of *Cl. sporogenes* and *Cl.* sp. (A), (from 2,000 to 500,000 spores per gram of cheese were used.) *Cl. pasteurianum* and *Cl.* sp. (A), which did not sporulate prolifically, were not included in this experiment as difficulty was experienced in obtaining a sufficiently heavy spore suspension. Since it was possible that pH prevented the development of spoilage by all of the clostridia as shown in columns 7 and 8, Table 3, pH determinations were therefore obtained on the three additional batches, namely, 5.30, 5.35 and 5.48. The results showed (Table 4) that the only spoilage produced was by *Cl.* sp. (B), in the batch at pH 5.48 and that spoilage was more pronounced with the higher inocula. These results indicated that low pH was a major factor in inhibiting spoilage regardless of the number of organisms present. However, where the pH was favourable, spoilage was much more pronounced with larger numbers. The failure of *Cl. sporogenes* to produce any spoilage may be accounted for by an unfavourable pH in the three batches of experimental process cheese.

C. *Effect of pH and Sodium Chloride on Clostridia in Laboratory Media*

The results of previous experiments indicated that the development of spoilage in process cheese could be controlled more easily by pH adjustment rather than by a reduction in the load of gas-producing clostridia. Since spoilage did not develop in the previous experimental batches at low pH values and since commercial process cheese contains sodium chloride which may act as an inhibitor, experiments were carried out to determine the effect of these two factors on growth and gas production by five clostridia. The organisms used were *Cl. sporogenes*, *Cl.* sp. (A), *Cl.* sp. (B), *Cl. pasteurianum*, all of which were capable of producing spoilage in experimental cheese, and *Cl. perfringens*, a prolific gas producer in anaerobic media.

To establish the effect of sodium chloride and pH on the behaviour of these organisms under optimum conditions, brain liver heart medium was

TABLE 4.—THE INFLUENCE OF NUMBER OF SPORES AND pH ON THE DEVELOPMENT OF SPOILAGE BY 2 SPECIES OF THE GENUS *Clostridium* IN EXPERIMENTAL PROCESS CHEESE HELD 8 DAYS AT 37° C.

Clostridium	Number of spores per gram	pH 5.30		pH 5.35		pH 5.48	
		Duplicate		Duplicate		Duplicate	
Cl. sporogenes	2,000	–	–	–	–	–	–
	10,000	–	–	–	–	–	–
	50,000	–	–	–	–	–	–
	500,000	–	–	–	–	–	–
	0	–	–	–	–	–	–
Cl. species (B)	2,000	–	–	–	–	++	+
	10,000	–	–	–	–	+++	+
	50,000	–	–	–	–	+++	+++
	500,000	–	–	–	–	++++	++++
	0	–	–	–	–	–	–

adjusted with lactic acid to a pH range of from 7.3 to 4.8. For each pH the sodium chloride content was adjusted to a final concentration of 0.5, 2.0 and 3.0 per cent. One drop of inoculum prepared by methods previously outlined was added to 10 ml. of brain liver heart medium in tubes containing gas vials. Incubation was at 37° C. for 4 days.

Table 5 shows the effect of pH and sodium chloride on the five clostridia in brain liver heart medium.

It will be noted that the salt concentration had no apparent effect on growth and gas production by the clostridia at the higher pH values as shown in Table 5. However, almost complete inhibition resulted at pH 5.0 or lower. At the intermediate pH values growth and gas production by *Cl. sporogenes*, *Cl.* sp. (B) and *Cl. perfringens* were either greatly diminished or completely inhibited by increased salt concentrations. On the other hand, growth and gas production by *Cl. pasteurianum* and *Cl.* sp. (A) were either initiated or vigorously stimulated by increased salt concentrations. It is apparent that, at the intermediate pH values, the salt concentrations used exerted opposite effects on these two groups of clostridia. Although the pH values above 5.0 and the salt concentrations below 3.0 per cent that completely inhibited growth and gas production varied with the organism, the results show that growth and gas production by clostridia can be controlled at practicable pH and salt levels.

D. Effect of pH and Sodium Chloride on Clostridia in Experimental Process Cheese

Further experiments were carried out to determine the effect of pH and salt on gas production by the five clostridia in experimental process cheese, with and without added casein digest. Experimental process cheese was prepared and inoculated by methods previously outlined for trials in reproducing the defect. In order to insure a uniform product, ingredients from the same sources were used for all batches. Each batch was adjusted with lactic acid or sodium hydroxide to a pH range of from 6.0 to 5.0. For each pH, the sodium chloride content was adjusted to a final concentration of 2.5, 3.0, 3.5 and 4.0 per cent. Incubation was at 37° C. for 8 days. The effect of pH, salt and casein digest on the five clostridia in process cheese is shown in Table 6.

It is evident from the results presented in Table 6 that no spoilage was produced by any of the organisms below pH 5.4 or at salt concentrations over 3.5 per cent above pH 5.4. No spoilage was produced by *Cl. perfringens* at any of the pH values or salt concentrations studied. Slight spoilage, which was not typical, was produced by *Cl. sporogenes* and *Cl.* sp. (B) at pH 5.4, 5.6 and 6.0. Increased salt concentrations inhibited gas production at these pH values (Table 6). The failure of these two clostridia to produce typical spoilage such as was produced in earlier experiments (Tables 3 and 4) indicated that these organisms were either subject to yet unrecognized factors in process cheese or possibly to mutation with a resulting loss of ability to produce large quantities of gas in process cheese. Typical spoilage was produced by *Cl. pasteurianum* and *Cl.* sp. (A) at pH 5.4, 5.6 and 6.0. At pH 5.4 some spoilage resulted when 3 per cent salt apparently overcame the inhibitory effect of pH. At pH 5.6 a salt

TABLE 5.—THE EFFECT OF pH AND SODIUM CHLORIDE CONCENTRATION ON GROWTH AND GAS PRODUCTION BY 5 SPECIES OF THE GENUS *Clostridium* IN BRAIN LIVER HEART MEDIUM INCUBATED 4 DAYS AT 37° C.

Organism	NaCl %	pH 7.3	pH 6.0	pH 5.8	pH 5.6	pH 5.4	pH 5.2	pH 5.0	pH 4.8
Cl. sporogenes	0.5	++++	++++	++++	++++	+++	+++	−	−
	2.0	++++	++++	++++	+++	+	−	−	−
	3.0	++++	++++	++++	+++	−	−	−	−
Cl. sp. (B)	0.5	++++	++++	++++	++++	++++	++++	−	−
	2.0	++++	++++	+++	+++	++	+	−	−
	3.0	++++	++++	++	+	+	−	−	−
Cl. pasteurianum	0.5	++++	++++	−	−	−	−	−	−
	2.0	++++	++++	+++	++	−	−	−	−
	3.0	++++	++++	++++	++++	−	−	−	−
Cl. sp. (A)	0.5	++++	++++	+	−	−	−	−	−
	2.0	++++	++++	++++	++	−	−	−	−
	3.0	++++	++++	++++	++++	−	−	−	−
Cl. perfringens	0.5	++++	++++	++++	++++	++++	++++	++	−
	2.0	++++	++++	++++	++++	++++	+	−	−
	3.0	++++	++++	++++	++++	++	−	−	−

++++ Very pronounced gas.
+++ Pronounced gas.
++ Definite gas.
+ Slight gas.
− Absent.

TABLE 6.—THE EFFECT OF pH AND SODIUM CHLORIDE ON GROWTH AND GAS PRODUCTION BY FIVE SPECIES OF THE GENUS *Clostridium* IN EXPERIMENTAL PROCESS CHEESE WITH AND WITHOUT CASEIN DIGEST, HELD 8 DAYS AT 37° C.

pH	NaCl %	Organism											
		Cl. sporogenes		*Cl.* sp. (B)		*Cl. pasteurianum*		*Cl.* sp. (A)		*Cl. perfringens*		Control	
		C.D.*	No C.D.	C.D.	No C.D.	C.D.	No C.D.	C.D.	No C.D.	C.D.	No C.D.	C.D.	No C.D.
5.0	2.5	−	−	−	−	−	−	−	−	−	−	−	−
	3.0	−	−	−	−	−	−	−	−	−	−	−	−
	3.5	−	−	−	−	−	−	−	−	−	−	−	−
	4.0	−	−	−	−	−	−	−	−	−	−	−	−
5.2	2.5	−	−	−	−	−	−	−	−	−	−	−	−
	3.0	−	−	−	−	−	−	−	−	−	−	−	−
	3.5	−	−	−	−	−	−	−	−	−	−	−	−
	4.0	−	−	−	−	−	−	−	−	−	−	−	−
5.4	2.5	+	+	+	+	−	−	−	−	−	−	−	−
	3.0	+	−	−	−	++	+	++	+	−	−	−	−
	3.5	−	−	−	−	−	−	−	−	−	−	−	−
	4.0	−	−	−	−	−	−	−	−	−	−	−	−
5.6	2.5	++	+	+	+	++	++	+++	++	−	−	−	−
	3.0	+	−	+	−	++++	++++	++++	++++	−	−	−	−
	3.5	−	−	−	−	+++	++	++++	+++	−	−	−	−
	4.0	−	−	−	−	−	−	−	−	−	−	−	−
6.0	2.5	+	++	+	+	++++	++++	++++	++++	−	−	−	−
	3.0	++	+	+	−	++++	+++	+++	++	−	−	−	−
	3.5	+	+	−	−	+++	−	+	−	−	−	−	−
	4.0	−	−	−	−	−	−	−	−	−	−	−	−

* 0.4 per cent added casein digest suspension.

++++ Very pronounced gas.
+++ Pronounced gas.
++ Definite gas.
+ Slight gas.
− Absent.

concentration of 3 per cent was most effective, 2.5 per cent and 3.5 per cent less effective in overcoming the adverse effect of pH. Apparently pH 6.0 had no effect on these organisms as increased salt concentrations inhibited the development of spoilage (Table 6). Spoilage in most instances was slightly more pronounced when casein digest was added.

COMMERCIAL METHODS OF CONTROL

The failure in every instance of *Cl. perfringens*, *Cl. tertium*, *Cl.* sp. (C) and *Cl.* sp. (D) to produce spoilage, when inoculated into experimental process cheese, showed that these organisms were incapable of development in experimental process cheese, and would therefore probably not be involved in outbreaks of spoilage in commercial process cheese. Likewise, since it has been shown that a high percentage of Cheddar cheese and skim milk powders contained clostridia and since trouble has been infrequently reported by processors who made no attempt to control this type of spoilage, it is probable that the majority of clostridia which are present in these ingredients are incapable of development in commercial process cheese. However, it is evident from the results of the foregoing experiments that some species of clostridia are capable of causing spoilage and that their development in process cheese can be controlled.

It is apparent that readily available nutrients, added in the form of casein digest, stimulated somewhat the development of spoilage at pH 5.4, 5.6 and 6.0 by *Cl. pasteurianum*, *Cl* sp. (A), *Cl. sporogenes* and *Cl.* sp. (B). The effect, however, was not pronounced, indicating that spoilage in commercial process cheese could not be prevented by controlling the nutrients alone.

Spoilage in experimental process cheese was completely controlled at pH values below 5.4, regardless of the salt concentrations, and at salt concentrations over 3.5 per cent, regardless of the pH. These results indicate that a salt concentration of approximately 4.0 per cent in conjunction with a pH approximating 5.2 can be used to control spoilage in commercial process cheese caused by gas-producing clostridia.

SUMMARY

1. Gas-producing clostridia were isolated from spoiled process cheese, spoiled process Blue cheese and suspected samples of Cheddar and Blue cheese.

2. An examination of 100 samples of skim milk powder and 51 samples of Cheddar cheese in anaerobic media for presumptive evidence of the presence of gas-producing clostridia showed clostridia in 64 per cent of the milk powders and in 92 per cent of the Cheddar cheese samples after 4 days' incubation at 37° C.

3. Typical but inconsistent spoilage was reproduced in experimental process cheese with *Cl. pasteurianum*, *Cl.* sp. (A), *Cl. sporogenes* and *Cl.* sp. (B). The defect could not, however, be reproduced in experimental process cheese with *Cl. tertium*, *Cl. perfringens* and *Cl.* spp. (C) and (D).

4. Low pH was found to be a major factor in inhibiting spoilage regardless of the number of organisms present. However, where the pH was favourable, spoilage was much more pronounced with larger numbers.

5. Spoilage in experimental process cheese was completely controlled at pH values below 5.4, regardless of the salt concentration and at salt concentrations over 3.5 per cent regardless of the pH.

6. Results show that a salt concentration of approximately 4.0 per cent in conjunction with a pH approximating 5.2 will control spoilage in commercial process cheese caused by gas-producing clostridia.

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AN OCCURRENCE OF ROT OF SPANISH ONION SEEDLINGS CAUSED BY *BOTRYTIS ALLII*[1]

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INTRODUCTION

Late in May, 1948, a vegetable grower at Leamington, Ontario, reported an unusually high mortality in his Spanish onion crop. The trouble was noted about 5 weeks after the seedlings were transplanted to the field. At that time, the crop was inspected by the writer who found that approximately 15 per cent of the plants were either dead or severely wilted and, of the remainder, about 20 per cent were chlorotic and unthrifty. Further surveys made at periodic intervals showed that the disease continued to spead and that the onions continued to die until mid-June. As a result of this trouble, the total loss in stand in the 8-acre field was estimated to be 40 per cent.

Brief mention of this seedling rot of onions in Ontario has already been made (3). The cause of the disease and certain factors influencing its development are reported in the present paper.

DESCRIPTION OF THE DISEASE

An examination of diseased specimens showed that the basal leaf tissue of the young plants was attacked slightly below the surface of the soil. The attack was initiated invariably at a point where one or more leaf blades arose from the central axis of the plant (Figure 1). The affected tissue was soft, water-soaked, and presented a cooked appearance. Although the part of the plant above the lesion was yellowed, and either wilted or dead, the roots and the leaf tissue below the lesion appeared to be healthy. The margin between the diseased and healthy tissue was quite definite. On the exterior of many lesions, a gray felt-like fungus growth was present. These symptoms resembled so closely those described by Ogilvie (5) and Walker (7) for neck rot of mature onion bulbs that, for verification of the identity of the disease, it seemed only necessary to isolate the causal organism.

ISOLATION FROM DISEASED PLANTS

Pieces of diseased tissue taken from the interior of the lesion were placed on plates of potato dextrose agar. Small amounts of fungus material growing on the exterior of the lesion were transferred to other plates of P.D.A. Without exception, a moderately fast-growing fungus developed from the tissue and the fungus plantings. Within 4 days the fungus produced abundant stout, brownish-walled, branched conidiophores that produced numerous hyaline, oblong to elliptical conidia, 4-8 μ wide by 6-15 μ long. At the end of 8 days, the surface of the culture presented a dense, gray-mould appearance. Thus, in cultural morphology, the fungus fitted very closely the description of the gray-mould neck rot organism, *Botrytis allii* Munn, given by Walker (7).

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For the purpose of comparison, an isolate of the gray-mould neck rot fungus was obtained from an onion bulb that developed rot in storage. These two isolates were grown separately at room temperature on a series of plates containing P.D.A. and cornmeal agar. The isolate obtained from the rotted bulb consistently produced a low dense mat of heavily sporulating mycelium, whereas the isolate from infected seedlings produced, in addition to the low mycelial mat, numerous small aerial tufts of grayish-white mycelium. In young cultures of the latter, conspicuously fewer conidia were produced than in those of the former, but in old cultures of both little difference was noted. In test tube slant cultures both isolates produced numerous appressoria. Sclerotia were only rarely produced in culture. During the 2-year period the disease was under investigation, the isolate obtained from the onion seedlings continued to produce conspicuously more aerial mycelium than the isolate obtained from the rotted bulb. Owen *et al.* (6) found that, when cultural variants of *B. allii* occurred, they reverted to the wild type after a few mass transfers.

LITERATURE REVIEW

Walker (7) reported that he had never found any of the 3 *Botrytis* species that caused neck rot of mature bulbs attacking the actively growing parts of the onion plant. He considered the neck rot organisms to be almost exclusively confined in their parasitism of onions to the attack of bulbs at harvesting or later in storage. He was, however, with considerable difficulty able to produce infection of green leaves with the 3 *Botrytis* sp. under conditions of excessively high humidity conditions in greenhouse experiments. In England (1), though the disease is again primarily a serious trouble of onions in storage, it has been reported to develop occasionally in the spring on the young bulbs of onions seeded in the autumn. It has also been reported to attack the base of seedlings either in the seed boxes or soon after planting in the field. In British Columbia (2), Woolliams has reported neck rot to attack the new growth from bulbs planted in the field in the early spring.

INFECTION EXPERIMENTS

Preliminary Tests of Pathogenicity

Since *B. allii* has been found to attack parts of young, growing onion plants only under rather exceptional conditions, it seemed necessary to determine the pathogenicity of the isolate obtained from the Spanish onion seedlings. Accordingly, an experiment was carried out during August, September, and October, 1948, in the following manner. Spanish onions were seeded at periodic intervals in flats of steamed soil and the seedlings were inoculated with *B. allii* when the basal leaf portion was about 4 mm. in diameter. After inoculation the seedlings were planted outdoors in flats of muck soil. Six separate series of inoculations, spaced from 6 to 12 days apart, starting on August 1 and terminating on September 17, were carried out. The following 3 methods of inoculation were performed in each of the 6 successive tests: (1) The basal leaf portion was dipped into an aqueous suspension of conidia before the seedlings were planted; (2) The basal leaf tissue was slightly bruised by rolling the seedlings between the

FIGURE 1. Onion seedling at right showing typical attack by *Botrytis allii*. Healthy seedling at left.

fingers prior to dipping them into the conidial suspension, and (3) A drop of the conidial suspension was allowed to fall on the basal leaf tissue after the seedlings were planted. Soil moisture was maintained at a high level during the course of the experiment. Final readings of the number of infected plants were taken on October 17. The details and results of this experiment are summarized in Table 1.

TABLE 1.—INFECTION OF SPANISH ONION SEEDLINGS WITH *Botrytis allii*

Date of inoculation	Number of plants infected			
	Method of inoculation			Check
	One drop of suspension after planting	Dipped into suspension	Bruised, then dipped into suspension	
August 1	0/24*	0/24	0/24	0/24
August 12	0/24	0/24	0/24	0/24
August 24	0/24	2/24	2/24	0/24
September 2	0/24	1/24	2/24	0/24
September 11	0/24	11/24	12/24	0/24
September 17	3/32	15/32	21/32	0/24

* In each fraction the numerator denotes the number of plants infected and the denominator, the number of plants inoculated.

As Table 1 shows, only the later inoculations resulted in infection. The inoculations made late in August and during September showed that the fungus attacked uninjured tissue and that there was no evidence to suggest that mild injury to the seedlings increased infection. There was a correlation between rate of seedling growth and the amount of infection. No attack occurred while the seedlings were growing rapidly, but infection occurred when their growth was slow. It was observed that all infections developed on the basal leaf tissue at the ground level or slightly below.

Effect of Temperature and Moisture Upon Infection of Onion Seedlings

A greenhouse experiment was set up in April, 1949, to determine the effect of soil temperature and soil moisture upon infection. For this experiment 3 Wisconsin tanks, each of 8-container capacity, were used—one at 15°, another at 20°, and the remaining one at 25° C. The containers were filled with muck soil and 15 onion seedlings, inoculated by dipping them into a conidial suspension, were transplanted into 6 of the 8 containers in each tank. Uninoculated seedlings were planted in the remaining 2 containers of each tank. In each tank, the soil in 4 of the cans (3 with inoculated and 1 with uninoculated plants) was maintained at a high level of moisture and in the remaining 4 cans at a moderate level of moisture. The results of this experiment are summarized in Table 2.

From the results presented in Table 2, it is apparent that as the temperature increased the amount of infection decreased, there being approximately 6 to 7 times as much infection at 15° as at 25° C. In this experiment, soil moisture appeared to have no obvious effect upon the

TABLE 2.—THE EFFECT OF TEMPERATURE AND MOISTURE UPON THE INCIDENCE OF INFECTION OF ONION SEEDLINGS BY *B. allii*

Soil temperature	Soil moisture	Infection in 25 days in per cent
15° C.	High	72
	Low	77
20° C.	High	45
	Low	51
25° C.	High	10
	Low	13

amount of disease that developed at any of the 3 temperatures. Noteworthy was the observation that seedling mortality appeared first at 15° C. and was progressively later at 20° and 25° C. No mortality was recorded later than the 25th day after the plants were inoculated, and at that time the uninfected onions were growing rapidly at the 3 temperatures. It was also observed that, within 8 days after inoculation, the fungus fruited abundantly on moribund or senescent leaf tissue that was covered with soil.

Other Pathogenicity Tests

In other tests designed to compare, both on seedlings and on mature bulbs, the pathogenicity of the isolate obtained from the onion seedlings with that of the isolate obtained from the rotted mature bulb, it was noted that both isolates were equally pathogenic.

DISCUSSION

Since this trouble hitherto has not been reported as being serious in the transplant onion crop in either Canada or United States, it would appear that rather precise conditions must obtain before infection can become established. In Canadian Plant Disease Survey (2), Woolliams reported a similar type of infection by *B. allii* as occurring in British Columbia in 1944 on the new growth from onion bulbs following a prolonged period of unusually cool weather. The results of certain infection experiments reported above showed that the disease developed only when soil temperatures were low.

In view of the facts that *B. allii* fruited readily on onion leaves that died after seedlings were transplanted and that the young plants became infected at a point where a senescent leaf blade joined the central axis, it would appear that under conditions of low temperatures, when the host was growing slowly, the fungus was able to attack healthy tissue after it had become established saprophytically on adjacent moribund tissue.

SUMMARY

Botrytis allii was observed to attack young onion seedlings in late May and in early June of 1948 in a field at Leamington, Ontario. Cultures of this organism were readily obtained from the affected plants. A cultural comparison of this fungus with a typical wild type isolate of the

gray-mould neck rot fungus showed only a slight morphological difference. Both isolates proved to be alike pathogenically. Infection and decay of young transplants were favoured by low soil temperatures; the highest mortality occurred at 15° C. and was progressively lower at 20° and 25° C.

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FRESH AND PROCESSED APRICOTS AS A SOURCE OF VITAMIN A[1]

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INTRODUCTION

Vitamin A, a fat soluble factor, is essential for normal health and development. The true vitamin is found only in animal tissue. Many plant tissues, however, contain antecedent substances or provitamins which exhibit vitamin A activity. These provitamins are often grouped under the heading "carotenoids" or simply "carotene". Of the sixty known carotenoids only four are known to possess vitamin A activity. Beta carotene which, because of its structure, yields theoretically two molecules of vitamin A, is the best source. The isomers alpha and gamma carotene, and cryptoxanthin, have approximately half the vitamin A value of beta carotene. Cryptoxanthin is not present in all plant foods but corn, for example, contains significant amounts. As the provitamin A substances in plant tissues are usually predominantly beta carotene, it is frequently used for calculating the vitamin A value of a plant food.

Amongst the fruits, apricots have been rated as one of the better sources of provitamin A. The results reported in this paper for fresh apricots are taken in part from a four-year study on a co-operative project with the British Columbia Fruit Growers Association (20). A comparison is made between the standard and new apricot varieties. Varieties grown in the Okanagan Valley are also compared with those grown in other areas. In addition, limited data are presented regarding the effect of processing and storage on the retention of provitamin A in processed apricots and apricot puree.

REVIEW OF LITERATURE

In a survey published prior to 1940, Fixsen and Roscoe (11) report the carotene content of fresh apricots to range from 1800 to 2300 mcg. per 100 gm. In 1941, values reported by Sherman (19) and recalculated to micrograms gave a probable range of 2400-4800 mcg. Lamb, Pressley and Zuch (15) report values ranging from 810 to 2140 mcg. and averaging 1590 mcg. A recent United States Government publication of tables of food composition (8) lists the carotene value for apricots as 1674 mcg. per 100 gm. Morgan (17) gives a mean value of 3030 and the average reported by Hewston and Marsh (13) is 2400 mcg. Baccari and Maranelli (5) found Italian-grown apricots to average 677 mcg.

In the preservation of fruits and vegetables by canning, the fat soluble vitamins probably are the least affected by the various operations involved. Cameron *et al.* (9) report apricots to retain from 77 to 98 per cent of their original provitamin A value, averaging 89 per cent.

The carotene content of canned apricots ranges from 930 to 1740 mcg. according to results published by Clifcorn (10) and Hinman *et al.* (14). The mean value found by the former investigator was 1280 mcg. and that

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of the latter was 1350 mcg. Lamb, Pressley and Zuch (15) report the provitamin A value of the canned fruit to range from 490 to 1240 mcg. with an average value of 800 mcg. per 100 gm. The retention reported by these investigators was similar to that of Cameron *et al.* (9), ranging from 78 to 98 per cent. Pressley, Ridder, Smith and Caldwell (18) give the carotene range for canned apricots as 930 to 1740 with an average of 1280 mcg. per 100 gm. On the assumption that all the carotene is present in the solids they calculate the fruit to contain from 1560 to 2900 with an average value of 2130 mcg. per 100 gm.

Guerrant *et al.* (12) conducted a comprehensive survey on a number of commercially processed foods from different regions in the United States. The product was analysed as soon after processing as possible and stored at temperatures of 50°, 65° and 80° F. Periodically during a 2-year storage period the samples were re-analysed. These investigators report a minimum retention value for apricots of 72 per cent. This figure was reached during the first 12 months at storage temperatures of 65-80° F. The carotene content of the canned apricots dropped to this value in almost a straight line and there was no further loss up to 2 years' storage. At 50° F. the loss was only 5 to 15 per cent.

In a similar experiment at storage temperatures of 70°, 90° and 100° F. Brenner *et al.* (7) found the carotene content to increase slightly up to 18 months. No significant variation between lots was noted due to differences of storage temperature. The carotene values reported for canned apricots by these investigators ranged from 820 to 1610 mcg. per 100 gm.

METHODS OF PROCEDURE

Harvesting

Fruit used in this experiment came chiefly from the orchards of the Summerland Experimental Station. Samples of each variety were harvested at various stages of maturity to simulate commercial pickings. The fruit was placed at 32° F. as soon as possible after harvesting and ripened at 60-70° F. and a relative humidity of 50 per cent. When the fruit was considered to be in good eating condition samples were taken for analysis and processing.

Processing

A number of hot (solid) pack samples were prepared as follows: The pitted fruit was heated to just under boiling and filled into 20-ounce plain cans at 200-212° F. After closing the cans were cooked in boiling water for 10 minutes, water cooled and stored at 55-60° F.

Samples of puree were prepared by heating and mashing the pitted fruit plus 5-10 per cent water in a stainless steel kettle at 190-212° F., extracting hot in a tomato juice expeller-extractor, re-heating to 190-200° F. and filling hot into 20-ounce plain cans. After sealing the cans were cooked in boiling water for 10 minutes, water cooled to approximately 110° F. and stored at 55-60° F.

Samples of the residue from the expeller-extractor were canned and stored for analysis.

Analysis

Carotene was determined chromatographically as beta carotene. Three hundred grams or more of the fresh or processed sample were placed in a Waring blendor for not more than 30 seconds to thoroughly macerate and mix the sample. Five or ten gram aliquots were blended for 5 minutes in alcoholic potash and extracted cold according to the method for fresh materials adopted by the A.O.A.C. (1, 3). The adsorption column proposed by Wall and Kelly (22) employing the use of three parts Celite and one part magnesium oxide was found to be the most satisfactory for chromatographic separation of the extracts under study and hence was used throughout this investigation in preference to the columns suggested by the A.O.A.C. Celite No. 503 was used. The extract was concentrated to 15 to 20 ml. in vacuo and the separation of the carotene fractions carried out according to the A.O.A.C. abridged chromtographic method (2). The carotene bands were eluted with 3 per cent and 10 per cent acetone in petroleum ether (4, 22), collected and determined in a Klett-Summerson photoelectric colorimeter using a combination of Corning filters No. 035 and 554. The instrument was previously standardized against solutions of purified beta carotene. Results were recorded as micrograms (mcg.) of carotene per 100 grams of material taken.

RESULTS

In Table 1 are presented the results of analysis of 49 samples of 11 varieties of fresh apricots as consumed. The distribution of carotene values for several varieties is given in Table 2.

The data show that apricots are very good to excellent in vitamin A value, ranging in carotene content from 700 to 4150 mcg. with an average of 1937 mcg. Marked varietal differences were noted. Wenatchee Moorpark, the leading commercial variety in British Columbia, had the lowest carotene content averaging 1207 mcg., with minimum and maximum figures of 700 and 2050 mcg., respectively. Sixty-five per cent of the samples

Table 1.—Carotene content of apricots

Variety	Number of samples	Carotene content in micrograms per 100 grams of fresh edible portion	
		Average	Range
		mcg.	mcg.
All varieties	49	1937	700 – 4150
Blenheim (Royal)	10	2225	1550 – 2800
English Moorpark	5	3490	2400 – 4150
Kaleden	4	1462	800 – 2050
Leslie*	1	1850	—
Perfection	2	2300	2100 – 2500
Reliable*	1	2450	—
Rose	2	2200	1750 – 2650
Tilton	9	1944	1500 – 2350
Wenatchee Moorpark	14	1207	700 – 2050
No. 27-5*	1	2150	—

* New varieties.

TABLE 2.—THE DISTRIBUTION OF CAROTENE VALUES OF COMMERCIAL APRICOTS

Variety	Number of samples	Percentage of samples in 200 microgram carotene classes											
		700-899	900-1099	1100-1299	1300-1499	1500-1699	1700-1899	1900-2099	2100-2299	2300-2499	2500-2699	2700-2899	Over 2900
Apricots—		%	%	%	%	%	%	%	%	%	%	%	%
All varieties	49	10.2	10.2	—	6.1	10.2	10.2	8.2	18.4	6.1	6.1	6.1	8.2
Blenheim (Royal)	10	—	—	—	—	20.0	—	—	40.0	10.0	10.1	20.0	—
English Moorpark*	5	—	—	—	—	—	—	—	—	20.0	—	—	80.0
Tilton	9	—	—	—	—	11.1	33.4	22.2	22.2	11.1	—	—	—
Wenatchee Moorpark	14	28.5	36.0	—	14.2	7.1	—	7.1	7.1	—	—	—	—

* Only variety analysed which exceeded 2900 mcg. per 100 grams. It is an old variety of limited commercial value.

contained from 700 to 1100 mcg. On the other hand, English Moorpark, a variety of rather limited production, was very rich in carotene, varying from 2400 to 4150 mcg. with most lots exceeding 2900 mcg.

Blenheim (Royal), Perfection, Rose and Tilton were all very similar in carotene content, ranging from 1500 to 2800 mcg. which makes these varieties approximately mid-way between Wenatchee Moorpark and English Moorpark in vitamin A potency. Eighty per cent of the Blenheim samples exceeded 2100 mcg. of carotene. Single sample determinations of the new varieties developed at the Experimental Station, Summerland, namely, Leslie, Reliable and No. 27-5 indicate these fruits have carotene contents similar to Blenheim and Tilton. The Kaleden variety was found to have the same values as Wenatchee Moorpark.

Retention of carotene in hot solid packed Blenheim, Tilton and Wenatchee Moorpark apricots ranged from 66 to 100 per cent, averaging 80 per cent. In the two samples of apricot puree, retention was only 43 and 61 per cent. The residue from these puree experiments contained 2900 mcg. and 2400 mcg. per 100 g. which is 32 per cent and 9 per cent more than was present in the fresh fruit.

DISCUSSION

Fresh Apricots

In carotene content, British Columbia apricots compare very favourably with fruit grown in other areas as reported by Fixsen and Roscoe (11), Booth (6), Sherman (19), Lamb, Pressley and Zuch (15), Morgan (17), Baccari and Maranelli (5) and are a rich source of this dietary constituent. The varieties, English Moorpark, Blenheim, Tilton and Perfection are an excellent source of provitamin A. The varieties, Kaleden and Wenatchee Moorpark, while lower in carotene content than the above mentioned varieties, are still a good source of the provitamin.

Hot Solid Pack Apricots

During this investigation, carotene retention in the hot solid pack fruit was slightly less than that determined by Cameron *et al.* (9), Lamb, Pressley and Zuch (15), and Guerrant *et al.* (12) for canned apricots. Retention values reported by the first two groups of investigators were computed on the dry weight basis. Those of the latter group were determined by comparison of results from analysis of the can's contents as a whole before and after storage. Moisture was not determined during this preliminary study and thus the results are not absolutely comparable with the above. However, indications are that, if they were computed on a similar basis, they would be in good agreement with those of the above investigators.

Although provitamin A is not water soluble or readily destroyed by heat, there is indication that the hot packing of fruit may result in considerable carotene loss. Working with sweet cherries, Strachan *et al.* (20) noted that this process reduced the carotene content to about one-half that of the fresh fruit. Just what percentage of the loss could be attributed to processing, and how much to chemical changes during storage, was not ascertained. It is interesting to note, however, that Mitchell, Van Blaricom and Roderick (16) reported a 52 to 54 per cent loss in the carotene content of peaches due to the cook.

The actual carotene values found for processed Tilton and Blenheim apricots were slightly higher than those reported by Clifcorn (10) and Hinman, Higgins and Halliday (14), and Pressley *et al.* (18). Values for Moorpark were similar to those reported by Lamb, Pressley and Zuch (15). It should be pointed out that the results of these investigators (15) are reported on a solid-liquid ratio of 60 : 40. Thus, while results of this study are not entirely comparable, indications are that they would be in very good agreement if calculated on the same basis.

Apricot Puree

In the literature there is a lack of data regarding the carotene content of purees and fruit juices. The fact that the two samples of puree analysed retained only 43 and 61 per cent of the carotene value of the fresh fruit indicates that considerable provitamin A is discarded in the refuse. This observation was borne out by the fact that analysis of the refuse showed large amounts of carotene present. Working with peaches, Mitchell, Van Blaricom and Roderick (16) found lye peeling resulted in a 10 to 15 per cent loss of carotene. The steam blanching of peaches in this laboratory resulted in a loss of 8 per cent. From the results of these investigations (16) together with the limited observation of this study, it appears that the amount of residue discarded is the chief contributing factor in carotene loss in the manufacture of a puree.

Dietary Vitamin A Value

The new dietary standard for Canada (21) sets the daily vitamin A maintenance allowance according to body weight for adults, body weight and age for children from 1 to 15 years, and additional allowances for pregnant and lactating women. For the purposes of evaluating the fruit as to its vitamin A value, an average figure of 5000 I.U. or 3000 mcg. of carotene was considered an average adult's daily requirement. A fruit contributing 50 per cent (1500 mcg.) of the value per 100 gram ($3\frac{1}{2}$ ounce) serving, was classified as an excellent source of vitamin A; 750-1500 mcg. a good source; 375-750 mcg. a fair source; and 0-375 mcg. a poor source.

Using the above basis of comparison, the fresh fruit of all the apricot varieties except Kaleden and Wenatchee Moorpark can be considered an excellent source of vitamin A. Twenty-one per cent of the Wenatchee Moorpark variety was in the excellent group. The remainder could be classified as good. Even after processing the hot packed fruit and puree is a good-to-excellent source of vitamin A.

SUMMARY

Apricots are an excellent source of provitamin A. High carotene content (vitamin A value) is closely related to variety. Even the varieties yielding the lower values supply considerable amounts of the provitamin.

While carotene is not readily destroyed during processing, commercial canning and hot solid packing of apricots with subsequent storage can result in a considerable lowering of the carotene content. In the manufacture of a puree the chief loss occurs in the amount of residue discarded.

Canned apricots should retain from 66 to 98 per cent and apricot puree approximately 50 per cent of the carotene originally present in the fresh fruit.

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RELATION BETWEEN TYPE AND BUTTERFAT PRODUCTION OF JERSEY COWS IN CANADA[1]

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The relation between type and butterfat production has often been investigated. The correlations found between type and production have usually been positive but small; e.g., of the order of 0.12 to 0.25. The present investigation concerned the relationship between type and single lactation records of butterfat in Jersey cattle. The specific questions investigated were: (*a*) the phenotypic correlation between type and butterfat production in the same cow; (*b*) the heritability of butterfat production; (*c*) the heritability of type, and (*d*) the genetic correlation between type and butterfat production.

SOURCE AND NATURE OF DATA

The data were taken from the First Consolidated Volume of the Testing and Classification Records of the Canadian Jersey Cattle Club. These included all cows that had qualified in the Canadian Record of Performance testing program and that had likewise been classified under the Canadian Jersey Herd Classification System between May 1941 and August 1946. Under the official testing program a breeder usually places all normal cows on test, although under the rules in force at the time of this study it was not compulsory to enroll all cows in the herd. The participation of the Jersey breeders in the Herd Classification program is voluntary. However, the regulations require that, if a breeder has any cows classified, he must have all cows classified that have freshened. The official inspectors classify each cow as: "Excellent", "Very Good", "Good Plus", "Good", "Fair" or "Poor". Since the registration papers of all cows classified as "Poor" must be surrendered, the "Poor" cows were not in the published records and could not appear in this study.

Only the butterfat record made nearest to five years of age was chosen, if more than one were available. All butterfat records were converted to a mature equivalent, 305-day, twice-a-day milking basis. For computation the type ratings were coded numerically as shown in Table 1.

For this study 3328 cows[2] with both an official type rating and an official butterfat record were available. These cows were by 1129 sires distributed throughout Canada. The daughters per sire averaged 2.95, but 578 of the sires had only one daughter each. The daughter-dam analysis consisted of 776 dams with 858 daughters. These daughters were sired by 360 sires. The percentages of the dams, of the daughters, and of all cows in each type classification group are shown in Table 1, along with the average butterfat production of each group.

The standard deviation of all type ratings as calculated from Table 1 was 0.92, which would reduce to 0.87 if Shepard's correction were made. For butterfat records the standard deviation was 75 pounds.

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[2] The total number of cows (3328) used in this study will be referred to as "all cows" throughout the article.

TABLE 1.—FREQUENCY DISTRIBUTION OF TYPE RATINGS WITH THE AVERAGE BUTTERFAT PRODUCTION FOR EACH CLASSIFICATION GROUP

Type rating	Coded score	Percentage of total			Average butterfat production		
		All cows	Daughters	Dams	All cows	Daughters	Dams
Excellent	6	6.7	7.0	11.1	480.6	486.0	484.5
Very Good	5	33.6	32.5	38.1	459.6	463.5	471.4
Good Plus	4	39.1	40.1	35.2	449.1	445.5	471.3
Good	3	17.6	17.2	13.0	448.8	440.7	451.0
Fair	2	3.0	3.1	2.6	449.8	473.9	445.5
Mean	—	4.23	4.23	4.42	454.7	454.2	469.5

PHENOTYPIC CORRELATION BETWEEN TYPE AND BUTTERFAT PRODUCTION

Phenotypic correlations were computed for all cows, for daughters and for dams. To remove the effect of sire differences on the correlation between type and production, the data were analysed on an intra-sire basis. In the case of the dams (which are also included in the "all cows") this analysis gives the average situation within groups of cows mated to the same sire. By thus removing the differences between sires, most of the genuine differences between herd averages (whether environmental or genetic in origin) probably were likewise removed. The intra-sire phenotypic correlations between a cow's type classification and her butterfat record for the lactation nearest five years, as shown from the statistics in Table 2, were + 0.13, +0.13 and + 0.21 for all cows, for daughters and for dams, respectively. These correlations are all significant statistically, although the difference between them is not.

The correlations of type and production between sires were only +0.05, +0.11, and 0.00 for all cows, for daughters and for dams, respectively. These correlations between sires are actually weighted correlations between the average production and the average type of the daughters by the same sire or, in the case of the dams, the averages of the group of cows mated to the same sire. The possible causes of such correlations are so numerous and complex that their interpretation is doubtful. They could arise from genetic effects or from purely environmental conditions which differ from herd to herd and affect production and appearance in the same direction, or from any combination of these. In these data the correlations between sires are so small as to be insignificant, or nearly so, and seem not to warrant further explanation. The groups by or mated to the different sires do differ genuinely from each other in average production and type. This is shown in Table 2, by the fact that the mean squares between sires are larger than the mean squares within sires. The difference is highly significant in all cases except for the production of dams. The magnitude of this group effect is shown by the intraclass "r" which shows how the members of the same group resemble each other in type or in production, as the case may be. Again, the interpretation is doubtful, since members of the same group were doubtless often classified on the same day and were usually kept and tested in the same herd. That the correlation is higher

TABLE 2.—ANALYSIS OF VARIANCE OF TYPE RATINGS AND BUTTERFAT PRODUCTION

Source of variation	d.f.	Type (T) mean squares	Production (P) mean squares	Covariance	r_{PT}
All cows—					
Total	3327	0.846	5660.3	6.441	
Between sires	1128	1.298	7715.5	5.046	0.05
Within sires	2199	0.615	4606.1	7.158	0.13
Intraclass r	—	0.27	0.19	—	
Daughters—					
Total	857	0.852	5859.0	8.551	
Between sires	359	1.184	6882.4	10.315	0.11
Within sires	498	0.612	5121.2	7.280	0.13
Intraclass r	—	0.28	0.13	—	
Dams—					
Total	775	0.876	7714.3	8.167	
Between sires	359	1.044	8099.5	0.087	0.00
Within sires	416	0.731	7381.9	15.292	0.21
Intraclass r	—	0.17	0.04	—	

among all cows and among daughters than among dams (who were only mates of the sire concerned, and not his daughters) may indicate that differences in transmitting ability of the various sires played a noticeable part in making their daughters somewhat alike in production and in type. Although this seems plausible and agrees with the other evidence, other interpretations are not sufficiently excluded to establish this one so securely that heritability could be estimated dependably from the closer resemblance of the daughters than of mates of a sire.

The intra-sire regression of butterfat production on type rating was 11.6 pounds. This estimate is very similar to those that have been reported by various investigations (1, 2, 6, 8). The figures for "all cows" in Table 1 indicate that the regression of butterfat production on type rating (ignoring sire differences) is not strictly linear. Table 1, if taken at face value, indicates that no correlation exists between type and production until type becomes better than "Good Plus", but it is not known whether any such non-linearity exists on an intra-sire basis. The apparent non-linearity in Table 1 could have been caused by prior selection of such a nature that cows of only "Fair" or "Good" type had generally been culled before classification unless their production was unusually high, whereas cows of type "Good Plus" or better had generally been retained regardless of production.

HERITABILITY OF TYPE

The heritability* of the differences in type ratings of the females mated to the same sire was computed by doubling the intra-sire regression of daughter's rating on dam's rating. In this study the regression coefficient

* Lush (5) has defined the degree of heritability as the fraction of the observed variance which is caused by differences in heredity. This fraction is a statistic, describing a particular population. It may vary from population to population for the same character and may vary from one character to another in the same population.

was 0.079 ± 0.049. This yields the estimate that about 16 per cent of the variance among type ratings of the mates of a sire is due to additively genetic differences; but this value is highly uncertain because the regression is based on only 498 degrees of freedom.

HERITABILITY OF BUTTERFAT PRODUCTION

The heritability of butterfat production was estimated in the same manner as that of type. The intra-sire regression of daughter's production on dam's production on a single record basis was 0.178 ± 0.043. Heritability of the differences among the mates of a sire in their butterfat records is then 0.36 with a standard error of about 0.09. This estimate of heritability is higher than most of the estimates that have been reported for the heritability of butterfat production.

GENETIC CORRELATION BETWEEN TYPE AND BUTTERFAT PRODUCTION

A genetic correlation may be defined as the correlation between an animal's genetic value for one character and the same animal's genetic value for another character. The genetic correlation between type and butterfat production was computed in this study from the four intra-sire covariance terms in Table 3. The geometric average of the two TP covariance terms could not be used, since they differed in sign. Hazel (3)

TABLE 3.—ANALYSIS OF COVARIANCE OF TYPE AND BUTTERFAT PRODUCTION FOR DAUGHTERS AND DAMS

Source of variation	d.f.	Crossproducts	Covariance
		T_D T_O	
Total	857	213.7	0.249
Between sires	359	184.9	0.515
Within sires	498	28.8	0.058
		P_D P_O	
Total	857	1,737,978	2028.0
Between sires	359	1,084,942	3022.1
Within sires	498	653,036	1311.3
		T_O P_D	
Total	857	5215.9	6.086
Between sires	359	2535.4	7.062
Within sires	498	2680.5	5.383
		T_D P_O	
Total	857	4629.3	5.402
Between sires	359	5181.3	14.433
Within sires	498	−552.1	−1.109

Where: T_O = Type of offspring (daughter). P_O = Production of offspring.
T_D = Type of dam. P_D = Production of dam.

recommended using the arithmetic average of the two covariance terms in the numerator in such cases. Since these two covariances are independent estimates of the same parameter, an arithmetic average can be used. Sampling errors seem likely to bias it less than they would the geometric average. The genetic correlation between type and production thus computed is,

$$\frac{2.137}{\sqrt{(0.058)\ (1311.3)}} = 0.245$$

This is slightly higher than the 0.18 obtained by Harvey (2). However, since the sampling errors associated with these estimates are large, the differences between the two estimates could easily have occurred just by chance.

DISCUSSION

The intra-sire phenotypic correlations between type and production, as found in this study were all highly significant statistically but are of only slight practical importance because they are numerically small. It appears, then, that within a herd only a slight amount of improvement could be expected in butterfat production by selecting for type alone. The correlation coefficient for all cows (0.13) is very similar to those reported by Harvey (2) with Jersey cows, Tyler and Hyatt (8) with Ayrshire cows, and Lush (6) with Holstein Friesian cattle, but it is somewhat lower than the correlation reported by Copeland (1), in a study of the type and production records of Jersey cows. The between-sire correlation of 0.05 is somewhat lower than the intra-sire coefficients. This indicates that among the daughters of a sire, type and production are more closely correlated than is the average type and average production of a sire group. The lower correlation between sires could have arisen from a difference among the selection programs of the breeders, wherein some strive for high production but pay only slight attention to type, while others pay more attention to type and less to production.

Heritability of type ratings as computed in this study was 0.16. This would indicate that heredity accounts for a real but small portion of the variation in type. This estimate is very similar to the 0.14 reported by Harvey (2) from an extensive study of Jersey cattle in the United States, but somewhat lower than the 0.30 found by Tyler and Hyatt (8).

The heritability of butterfat production in this study is high in comparison with those that have been found in most of the previous studies. A heritability of 0.36 is similar, however, to the highest values reported by Plum (7) and by Johansson and Hansson (4). It is possible that the genetic variance for butterfat production was actually higher in these data than in some of the other sets. Should heritability of butterfat production actually be this high, selection for production through a testing program would result in considerable improvement in each generation of dairy cattle. The genetic correlation of 0.245 between an animal's type and its butterfat production, as found in this study, is considerably higher than the intra-sire phenotypic correlation of 0.13. This latter correlation is actually a sum of a genetic portion, and a portion caused by at least some of the environmental variations affecting type and production in the same direction. If environment is defined as all causes of variation which are not

included in the estimate of heritability, it appears that the observed correlation (0.13) comes more largely (0.076) from environmental effects pushing type and production in the same direction than from genetic correlation (0.058).

SUMMARY

The data consisted of the official type classifications and official butterfat records of 3328 Jersey cows in Canada. The daughter-dam analysis included 776 dams with 858 daughters sired by 360 sires.

The average butterfat production of all cows, corrected to a mature equivalent, 305-day, twice-a-day milking basis, was 454.7 pounds with a standard deviation of 75 pounds. The average type rating of all cows was in the upper part of "Good Plus" with a standard deviation of 0.92. The probably few cows classified "Poor" had been excluded automatically from this study, as their registry was cancelled as soon as they were classified "Poor".

Heritability of butterfat production and over-all type score were computed by doubling the intra-sire regression of daughter on dam. The portion of the differences in type ratings that is expected to be transmitted from parents to offspring was calculated as being 16 per cent. The differences in butterfat production were estimated to be 36 per cent heritable. Both estimates, however, contain large sampling errors.

The intra-sire phenotypic correlations between type and a single butterfat record are + 0.13, + 0.13, and + 0.21, for all cows, for daughters, and for dams, respectively. These values are all highly significant, although the differences between them are not statistically significant.

The correlation between the genotype for type and the genotype for butterfat production, as calculated from the four covariances in this study, was + 0.245.

The intra-sire regression of butterfat production on type rating was 11.6 pounds. This means that the average production is expected to increase 11.6 pounds for each increase of one class in type rating. This 11.6 is about one-sixth of the intra-sire standard deviation in butterfat production.

ACKNOWLEDGMENTS

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THE EMBRYO TEST AS A METHOD OF FORECASTING LOOSE-SMUT INFECTION IN BARLEY[1]

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The embryo test, described by Simmonds (3) and Popp (1), enables one to detect the mycelium of *Ustilago nuda* (Jens.) Rostr. in the embryos of barley seeds. It is often very helpful to know whether or not a sample of seed is infected with loose smut. If, at the same time, it can be learned what percentage of the embryos carry the disease, the information is all the more useful. Purchasers of registered and certified seed should know if any loose smut is present in the stock and, if so, what percentage of the subsequent crop is likely to be smutty, because they pay a premium for this high quality grain. A knowledge of the extent of loose-smut infection may determine whether or not the seed should be treated.

In the past, the percentage of smutted heads in the parent crop has been accepted as a gauge of the amount of loose smut that the seed carried. Occasionally, growth tests were made before sowing the bulk of the seed. Neither of these methods is entirely satisfactory. The field count of the percentage of smutted heads may give an erroneous idea of the percentage of infected plants that will develop from the seed of that crop. There are two main reasons for this. In the first place, the amount of smut in the new crop is determined largely by weather conditions at the time the parent crop is in flower, and by the number of loose smut spores present in the air at that time. If weather conditions at that critical period are very favourable for infection, and if spores are plentiful, the new crop may carry a higher percentage of infection than was present in the parent crop; if unfavourable, and if spores are few, it may carry a lower percentage. In the second place, if false loose smut (*Ustilago nigra*) is present in the parent crop it may be mistaken for true loose smut and the count may be unduly high. There are also minor sources of error. For example, if the field count is made after the spores have been shed, it is sometimes very difficult to see all of the bare rachises of the diseased heads; or, if any roguing has taken place, the field count may be too low.

Growth tests give a good indication of the amount of loose smut carried in the seed, but such tests require considerable time. If these tests are conducted in a greenhouse in the winter, so as to secure the information before the next year's crop is to be sown, it is sometimes difficult to get sufficient space to grow an adequate number of plants. Several hundred plants of each sample must be grown if dependable results are to be secured (2).

The embryo test provides a quick and easy way of determining how much loose smut is carried by any sample of barley. This test can be applied to the seed as soon as it is threshed. By the use of this method,

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TABLE 1.—RESULTS OF A STUDY OF *Ustilago nuda* IN BARLEY. COMPARISON OF PERCENTAGES OF INFECTION AS SHOWN BY FIELD INSPECTION 1949, EMBRYO TEST OF THE 1949 CROP, AND GROWTH TEST OF THE 1949 CROP

Sample number	Province of origin	Percentage infection				
		Field inspection	Embryo test of 1949 crop		Growth test of 1949 crop	
			Saskatoon	Winnipeg	Saskatoon	Winnipeg
1	Alberta	0.01	0.3	0	0.3	0
2		0.04	0.5	1.2	1.1	0.5
3		0.20	3.4	3.0	2.4	4.6
4		0.10	0.7	0.9	0.5	0.5
5		0.20	2.4	4.4	3.8	5.4
6		0.40	0	0.4	0	0.4
7		0.40	2.3	4.6	1.6	5.4
8		0	0.2	0.2	0	0.4
9		0	0.9	2.4	1.1	1.6
10		0	0.9	0.6	1.7	0.6
11		0.67	1.6	2.0	1.6	2.5
12		9.57	4.6	5.8	5.5	7.0
13		1.58	0.6	1.6	0.5	1.1
14	Manitoba	0	0	0	0	0
15		0	0.2	0	0.3	0
16		0	0	0.6	0.3	0.5
17		0.02	0.7	1.0	0.7	0.2
18		0.28	0.6	0.4	0	0.4
19		0.05	0.1	1.2	0.9	0.2
20		0.27	0.6	0.5	0.8	0.2
21		0.68	2.3	1.8	1.3	2.2
22		0.03	0.9	2.0	1.9	2.3
23		0.78	1.2	1.8	1.9	1.9
24		0.80	1.5	0.4	0.9	0.4
25		0.17	3.4	1.6	3.4	2.9
26		0.07	0.1	0.6	0.2	0.6
27		0.70	0.3	0.4	1.4	1.8
28	Ontario	1.05	0	0	0.4	0
29		0	0	0	0	0
30		0	0	0	0.1	0
31		0	0.3	0.6	0.5	1.0
32		0.05	0	0	0	0
33		0.32	4.9	7.0	4.8	4.2
34		0	0.3	0	0.4	0
35		0.03	0	0.2	0.2	0.2
36		0.02	0.7	0.6	0	0
37		0	0.1	0.4	0.1	0.4
38		0	1.3	2.0	1.4	1.5
39		0.03	0.5	1.0	0.4	0.4
40		0	0	0	0	0
41		0.03	1.4	0.4	0.5	0.2
42		None reported	8.8	11.6	8.0	10.0
43		0.70	1.7	2.0	1.0	0.5
44		0.70	7.5	10.8	7.7	8.8
45		1.20	3.5	5.8	2.2	3.5
46		0.80	5.6	9.8	6.0	5.3
47		1.80	1.5	1.6	0.9	0.8
48		0.90	1.0	2.0	0.4	0.9
49		1.20	1.0	2.0	1.1	1.6
50		0.90	7.2	7.8	10.5	6.4
51		0.70	3.5	3.6	2.0	2.6
52		2.10	6.7	10.2	7.1	7.5
53		1.00	0.9	1.4	2.0	2.4

TABLE 1.—RESULTS OF A STUDY OF *Ustilago nuda* IN BARLEY. COMPARISON OF PERCENTAGES OF INFECTION AS SHOWN BY FIELD INSPECTION 1949, EMBRYO TEST OF THE 1949 CROP, AND GROWTH TEST OF THE 1949 CROP—*Concluded*

Sample number	Province of origin	Percentage infection				
		Field inspection	Embryo test of 1949 crop		Growth test of 1949 crop	
			Saskatoon	Winnipeg	Saskatoon	Winnipeg
54	Ontario	0.90	8.8	5.8	5.6	4.3
55	*(con.)*	1.00	1.1	1.6	2.8	2.0
56		1.35	10.5	9.2	5.6	10.4
57		1.10	6.5	6.8	6.3	6.2
58	Quebec	0	0	0	0	0
59		0	0.6	0.2	0	0.4
60		0	0.3	0	0.9	0.4
61		0	0.2	0	0	0
62		0	0	0.6	0.1	0.2
63		0	0	0	0	0
64		0	0	0.2	0	0
65		0	0	0.2	0.2	0.5
66		0	0.7	0.4	0.4	0.8
67		0	0.6	0.2	0	0
68		0	0.6	0.6	0	0.2
69		0	0.6	1.2	0.9	0.7
70		0	1.0	2.2	0.4	2.1
71	Saskat-	0	0.7	0.2	0.6	0
72	chewan	0	6.9	5.6	8.5	7.9
73		0.20	0.9	0.6	0.9	1.0
74		0	0.4	0.4	0.4	0.2
75		0	0.5	0	0.1	0
76		0.30	0.1	0.4	0.3	0.2
77		0	2.7	3.8	2.7	4.1
78		0.30	0.2	0.2	1.6	0.4
79		0.20	2.0	2.0	2.4	1.5
80		8.90	0.7	1.0	0.9	0.2
81	Prince	0.02	0	0.2	0	0
82	Edward	0	0	0	0	0
83	Island	0	0	0	0	0
84		0	0	0	0	0
85		0	0	0	0	0
86		0	0	0.2	0.1	0
87		0	0	0	0.06	0
88		0	0.2	0	0	0
89	New	0	0.2	0	0.2	0.2
90	Brunswick	0	0	0	0	0
91	Nova Scotia	0	0	0	0	0
Average infection		0.49	1.49	1.80	1.47	1.60

Russell (2) and Popp (1) secured very good agreement between the percentage of infection in barley seed, and the percentage infection that appeared in the field when other portions of these samples were grown to maturity. According to Popp (1), the embryo test has certain limitations as far as wheat is concerned, because the final result is affected not only by the percentage of infection found in the seed, but also by the variety of the

host and the physiologic race of the smut. So far, in barley, there is no indication that these factors interfere with the close agreement between embryo tests and growth tests when dealing with naturally inoculated seed.

MATERIALS AND METHODS

The material used in this investigation consisted of samples of barley from fields that were intended for the production of certified or registered seed. Ninety-one samples were obtained from offices of the Plant Products Division of Production Service, Canada Department of Agriculture, located in six different provinces across Canada. Each sample was divided into two equal parts at the Dominion Laboratory of Plant Pathology, Saskatoon. One lot of samples was kept at the Saskatoon Laboratory and the other lot was sent to the Dominion Laboratory of Plant Pathology, Winnipeg. Embryo tests and growth tests were made at the two laboratories on each of the samples. Simmonds' method of preparing and examining the embryos was used at Saskatoon, and Popp's method was used at Winnipeg. At Saskatoon about 600 embryos from each sample were examined and approximately 1000 heads were grown in 1950 from seed of each sample in small field plots replicated five times. At Winnipeg about 500 embryos from each sample were examined, and two small field plots were sown in 1950 with seed of each sample, each pair of plots producing approximately 450 heads. When the plants were in head, counts were made of the total number of heads and the number of smutted heads in the plots of each sample. A record of the amount of smut present in the parent crop of each sample was secured from the Plant Products Division.

RESULTS

The percentage of loose smut present in the parent crop as determined by field inspection in 1949, the percentage of infected embryos in the seed of the 1949 crop, and the percentage of smutted heads in plots grown from this seed are shown in Table 1 for each of the samples.

As shown in Table 1, the average infection found in the standing crop, from which these samples of seed came in 1949, was 0·49 per cent. The results of the embryo tests conducted at Saskatoon indicate that the average infection in seed produced in 1949 was 1.49 per cent. The average infection in the standing crop produced from these seed samples in 1950 at Saskatoon was 1.47 per cent. According to the results secured at Winnipeg, the 1949 seed showed smut in 1.8 per cent of the embryos and the standing crop in 1950 showed 1.6 per cent of the heads to be infected.

A close study of the table shows that the amount of infection reported by the field-crop inspectors in 1949 often differed widely from the amount found in the next generation of the same lines in the field plots in 1950. There were several instances of a substantial increase in smut infection, e.g., numbers 5, 22, 25, 33, 44, 50, and 72 and one instance of a decided decrease, i.e., number 80. In the majority of cases, the embryo counts agree better than the 1949 field counts with the results of the growth tests in 1950.

Not only did the embryo test, in most instances, give a better indication of smut infection than the inspectors' counts, but the deviations between the percentages obtained by the embryo tests and the growth tests were

not so great as those between the inspectors' counts and the 1950 fiel counts. For example, the maximum deviation between the embryo tests and growth tests was 4.9, while the maximum deviation between the inspectors' counts and growth tests was 10.1.

The extent of agreement between the results obtained by the different tests may be expressed mathematically by giving the following coefficients of correlation. First, with the results secured at Saskatoon, the coefficient of correlation between the percentages of infection recorded in the field in 1949 and in the field plots in 1950 is 0.23. Between the percentages obtained by means of the embryo test and the 1950 field-plot results, it is much better, namely 0.92. The corresponding coefficients of correlation from the data secured at Winnipeg are 0.27 and 0.94. Finally, the coefficient of correlation between the results of the embryo tests made at Saskatoon and those made at Winnipeg is 0.93. The second, fourth, and fifth of these coefficients are highly significant. On the other hand, the third is barely significant to the 1 per cent level, and the first is significant to the 5 per cent, but not significant to the 1 per cent level.

Additional Tests

In addition to the 91 samples investigated as described above, the senior author tested 20 samples of barley seed produced in 1949 on farms and on experimental plots in Saskatchewan. A comparison was made between the percentage of loose smut found in the embryos of these samples and the percentage of loose smut that developed in rows grown in experimental plots in 1950 from portions of the same samples. No records were available concerning the amount of smut in the parent crop in 1949. The embryo test involved about 500 embryos of each sample and the percentages obtained in the growth test are based on approximately 1100 heads in each case.

TABLE 2.—COMPARISON OF THE PERCENTAGE INFECTION BY EMBRYO TEST AND BY GROWTH TEST OF 20 SAMPLES OF BARLEY PRODUCED IN SASKATCHEWAN IN 1949

Sample number	Variety	Percentage infection	
		Embryo test	Growth test
1	Titan	7.1	7.7
2	Montcalm	0.6	0.2
3	"	0.6	0.3
4	"	0.6	0.3
5	"	0.6	0.6
6	?	0	0.3
7	?	9.4	9.9
8	Montcalm	0	0.7
9	"	2.8	3.0
10	"	0.2	0.3
11	Titan	0	0
12	Plush	2.5	3.0
13	Montcalm	0.2	0.1
14	?	0	0
15	Montcalm	0	1.0
16	"	0	0.2
17	"	3.0	1.2
18	Newal	2.0	0.9
19	Rex	1.6	1.0
20	Newal	6.6	5.6
Average infection		1.89	1.81

The coefficient of correlation between the percentages of infection, as determined by the embryo test, and the percentages of infection that actually developed in the field was 0.97.

DISCUSSION

The results of the main experiment indicate that inspection reports are less reliable than embryo tests as a means of forecasting the amount of loose smut that will develop in the succeeding barley crop. The embryo test is not an exact method of forecasting the amount of smut, but if the sample tested is truly representative of the seed lot in question, it enables one to make a close estimate of the percentage of smut that will appear in the crop from any given seed lot.

It is very important that all samples intended for testing should be taken in such a manner that they will be truly representative of the whole seed lot. Experience has shown that the percentage of infected embryos in different samples, taken from several different parts of a seed lot, may vary considerably, especially if the samples are small. Therefore, a sample selected for an embryo test should be composed of many small portions taken from different parts of the seed lot and thoroughly mixed together.

In the main investigation, it is possible that the predictions would have been more accurate if more embryos had been examined. In some instances, the embryo test showed no infection but a few smutted heads appeared in the growing crop. However, the examination of more embryos would require proportionately more time and effort and might be impractical when making routine tests. It takes from 30 to 50 minutes to examine 600 embryos after they have been prepared for examination. The amount of time required to prepare each sample depends somewhat on the quality of the seed, but with good, plump, uniform seed, it was found that the average time devoted to each sample was about 80 minutes. With additional experience and improvements in technique, it may be possible to shorten the time required for the test. The entire test, including examination of the embryos, can be performed by a technician.

In the work reported here, there are other possible sources of error, apart from the number of embryos examined. The number of heads were counted instead of the number of plants in the 1950 field-plot tests. If, on the average, diseased plants produce either more tillers or less tillers than healthy plants, this will constitute one source of error. If diseased embryos are slightly weaker than healthy ones, so that a smaller percentage of them germinate, as suggested by Wells and Platt (4), that will constitute an additional source of error. Possibly in some instances healthy plants develop from infected embryos through the failure of the fungus to keep pace with the development of the growing point. In all instances in Tables 1 and 2, the average infection, as determined by the "growth test", is slightly less than that determined by the "embryo test". It is evident, however, that none of the factors mentioned above had a very marked effect on the final results; otherwise the agreement between the results of the embryo test and the growth test could not have been so close.

The embryo test should prove to be of practical value, particularly to plant breeders and seed growers. By means of it, plant breeders would be able to determine, as soon as the grain is threshed, how much loose-smut infection is present in any variety or line of barley with which they were concerned. Providing the tests were made available to seed growers, they could use the results of the embryo test for the purpose of selecting disease-free seed, or for deciding whether or not the seed required treatment with hot water.

SUMMARY

Ninety-one samples of barley seed were secured from officers of the Plant Products Division, Canada Department of Agriculture, in six provinces. Half of each sample was studied at Saskatoon and the other half at Winnipeg.

The percentage of loose smut in the standing crop in 1949, the percentage of infected embryos in each sample produced in 1949, and the percentage of loose smut that appeared in experimental plots grown from these samples in 1950 were compared.

It was found that there was a high correlation between the results of the embryo tests and the field plot tests at each place. On the other hand the percentages of loose smut found in the growing crop in 1949 did not agree very closely with the percentages found in the experimental field plots in 1950.

It is suggested that the embryo test might be used to advantage by plant breeders, officials responsible for seed certification, and seed growers.

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BOOK REVIEW

TRACE ELEMENTS IN PLANT PHYSIOLOGY; A symposium organized by the International Union of Biological Sciences at the Rothamsted Experimental Station with a Report of the Proceedings by T. Wallace. Pages XVIII + 144. The Chronica Botanica Company, Waltham, Mass., and the Wm. Dawson Subscription Service, Ltd., Toronto. 1950. Price $4.50.

This symposium, held in 1947, furnished the first post-war opportunity for workers to discuss developments during the war. Contributors included leading scientists from Sweden, Finland, Netherlands, Denmark, France, Czechoslovakia, Great Britain and the United States. The main topics refer to methods of studying the rôle of trace elements in plant physiology and to practical problems encountered in crop production.

All contributions are in English, with the exception of Professor Lavollay's paper, which is in French. They are well written and in a readable style which should appeal to both specialists and to general agriculturists. Each paper is followed by highlights from its discussion, a most commendable practice.

It is particularly interesting to learn that all the represented countries have problems in mineral nutrition and are taking therapeutic measures while at the same time carrying out research relating to the rôle of such elements as boron, manganese, copper, zinc and molybdenum in the metabolic processes of plants. A paper by Prof. Seekles deals with trace element problems in farm animals and shows the importance of plant nutrition studies in relation to animal and human nutrition.

In a symposium of this nature it is impossible to organize the papers to deal separately and in logical sequence with recognized aspects of trace element nutrition. The reader is left the task of co-ordinating related studies and sorting conflicting views and experimental results. Nevertheless, the information available will be useful to those engaged in research on plant and animal nutrition.

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